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TITLE: **Enhanced Wound Healing Using Topically Administered Nanoparticle Encapsulated siRNA**

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14. ABSTRACT
The ongoing engagement of US troops in global combat activity has increased the incidence of complex wounds resulting in morbidity, permanent disfigurement, progressive disability, and/or chronic pain. Our long-term objective is to improve the quality of healing of wounds by providing military personnel with a therapeutic that can be immediately applied topically to damaged tissue to enhance wound closure, re-vascularization and appropriate re-innervation. This will save lives, but also vastly improve the quality of lives for those soldiers who survive with injuries.
The specific objective of the work supported by this award was to develop nanoparticle-encapsulated siRNA technology that can be topically administered to complex wounds to stimulate healing by altering cell motility and morphogenesis. Toward this end, we identified set of 4 genes encoding regulators of the microtubule cytoskeleton that can be targeted by nanoparticle-delivered siRNA to selectively enhance wound re-epithelialization, axon regeneration and blood vessel formation.

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The ongoing engagement of US troops in global combat activity has increased the incidence of complex wounds resulting in morbidity, permanent disfigurement, progressive disability, and/or chronic pain. Our long-term objective is to improve the quality of healing of wounds by providing military personnel with a therapeutic that can be immediately applied topically to damaged tissue to enhance wound closure, re-vascularization and appropriate re-innervation. This will save lives, but also vastly improve the quality of lives for those soldiers who survive with injuries.

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The Specific Aims of this research project were:

1) To determine the cell biological impacts of nanoparticle-siRNA mediated protein knockdowns using in vitro and ex vivo models.

2) To establish the efficacy of nanoparticle-siRNA treatments on wound regeneration in vivo (Preclinical studies).

3) To optimize the nanoparticle-siRNA delivery platform.

Research was performed by an interdisciplinary research team housed primarily at the Albert Einstein College of Medicine with combined expertise in cell/molecular biology, nanotechnology, dermatology, neuroscience and cardiovascular development. Through the support of this award, we have been able to establish novel paradigms to promote various forms of tissue regeneration and repair including regeneration of cutaneous excision wounds and burns, cardiac tissue following ischemic insult, and neural trauma (i.e. spinal cord injury). The ease with which our platform is generated, its low cost, and its simplicity of application in the field of battle all make it an ideal technology for military needs.

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1. **INTRODUCTION:**
   The goal the research supported by this award was to develop topical wound healing therapeutics suitable for battlefield use. Toward this end, we have generated a platform of nanoparticle-encapsulated siRNAs that target specific regulators of the microtubule cytoskeleton to promote tissue regeneration and repair by altering intrinsic cellular motility. Military skin and soft tissue wounds are often devastating and complex. They can encompass a large surface area involving multiple limbs and become contaminated by dirt or other foreign bodies. The immunity of injured soldiers is also compromised due to breaks in the skin. This combination of serious complications and other concomitant injuries such as intra-abdominal or head trauma, demands extensive and continuous wound management from the time of injury throughout the healing process.

2. **KEYWORDS:** Microtubules, nanoparticles, siRNA, cell migration, Fidgetin, Fidgetin-like 2, Cep192, Kif19, wound healing, revascularization, neural regeneration

3. OVERALL PROJECT SUMMARY:

Task	Q1	Q2	Q3	Q4	Status
Q1T1					**Completed**
Q1T2					**Completed**
Q1T3					**completed**
Q1T4a					**Completed**
Q1T4b					**Completed**
Q2T1					**Completed**
Q2T2					**Completed**
Q2T3					**Completed**
Q2T4					**Completed**
Q2T5a					**Completed**
Q25b					**Will not be performed**
Q3T1					**Completed**
Q3T2					**Completed**
Q3T3					**Completed**
Q3T4					**Completed**
Q3T5					**Completed**
Q4T1					**Completed**
Q4T2					**Completed**
Q4T3					**Completed**
Q4T4					**Completed/initial manuscript will be submitted shortly**

FY12 Quarter 1 Task 1. Generate usable quantities of the existing np-si formulation
This has been completed

FY12 Quarter 1 Task 2. Initial nanoparticle optimization (size distribution and cationic properties)
This quarter, optimization studies on the siRNA nanoparticle delivery platform progressed along two parallel trajectories: one focusing on optimizing cell uptake while maintaining siRNA functionality; and the other addressing the homogeneity and stability of the nanoparticles when suspended in a liquid delivery medium.

Attempts at optimizing cell uptake focused on modifying the overall charge on the nanoparticles. There are suggestions from the literature that cationic nanoparticles tend to be taken up cells more readily than either neutral or anionic nanoparticles. Our strategy for inducing cationic properties into our platform entailed doping our initial and standard tetramethoxysilane component of the nanoparticle formulation with amine containing silanes. N-(2-aminoethyl)-11-aminoundecyltrimethoxysilane (AUTS) was used to introduce two amines per added silane dopant molecule and aminopropyltriethoxysilane (APTES) was used to introduce a single amine

per added silane dopant molecule. Both additions yielded well behaved nanoparticles with physical properties comparable to the undoped nanoparticles. The added amines imparted cationic character to the particles. In preliminary cell uptake studies, the amine containing nanoparticles appear to adhere to the cell surface. Follow up studies are being planned to assess whether the amine particles merely stick to the cell surface or actually enter the cells. In a related study addressing passage of the nanoparticles through a blood brain barrier (BBB) model system, it was similarly observed that the amine containing nanoparticles stuck to the endothelial cells and did not effectively pass through the BBB whereas the neutral nanoparticles did pass through. PEGylation of the amine loaded nanoparticles enhanced passage through the BBB and as a result will also be evaluated with respect to cell uptake and siRNA activity.

Optimization of the suspension properties of the nanoparticles is a high priority since time dependent aggregation can complicate both quantitative comparative assays of functional properties and product development. The approach being pursued is two pronged. The first strategy focuses on a modification to the preparative platform that utilizes alcohols and much less water in an attempt to make the particles smaller and more uniform. This approach appears to be working and once the protocol is established, siRNA loading will commence with concomitant functional assays as well as cell uptake evaluation. The second approach for improving that stability of the nanoparticle suspensions is to PEGylate the nanoparticles subsequent to siRNA loading, Current efforts are focused on devising the best PEGylation protocol that allows for retention of the loaded siRNA during the PEGylation process. As noted above PEGylation of the "empty" particles improves BBB crossing as well as dramatically improving the stability of the suspended nanoparticles. In that study, PEG 2000 with a terminal methoxy group was used to coat amine loaded nanoparticles.

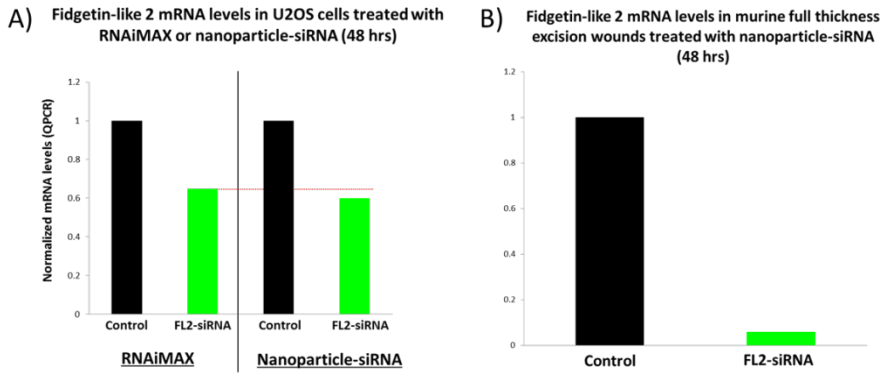


Figure 1: QPCR analysis of nanoparticle siRNA-mediated knockdowns in vitro and in vivo

FY12 Quarter 1 Task 3. Optimize nanoparticle treatment protocol in vitro.

As mentioned above, we have encountered a technical issue related to the degradation of our antibodies to Fidgetin, Fidgetin-like 2 and Kif19 which has hindered this task. However, we have completed a first run optimization of

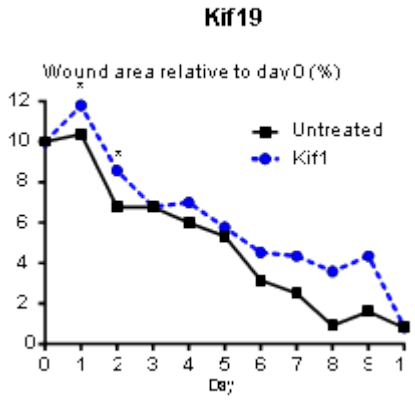


Figure 2: Kif19 nanoparticle-siRNA treatment attenuates wound closure

our treatment protocols by comparing mRNA knockdown levels (monitored by QPCR) following nanoparticle siRNA treatment vs. siRNA transfection using the RNAiMAX delivery system from InVitrogen (http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Protein-Expression-and-Analysis/Transfection-Selection/lipofectamine-rnaimx.html). RNAiMAX is the gold standard for siRNA transfection in tissue culture cells. We have found that human U2OS cells plated in culture media containing a final concentration of 200 nM Fidgetin-like 2 nanoparticle-siRNA display a knockdown of Fidgetin-like 2 mRNA that is comparable to RNAiMAX treatments (Figure 1A). Peak knockdown is observed at 48 hours after treatment with mRNA concentrations often returning to pre-treatment levels by 72 hours. Similar results have been obtained for Cep192, Kif19 and Fidgetin (not shown). These findings will be confirmed in February upon receipt of our new monoclonal antibodies. On a side note, we have also generated strong knockdowns of target mRNAs in vivo and ex vivo (surface wounds and embryonic hearts; Figure 1B).

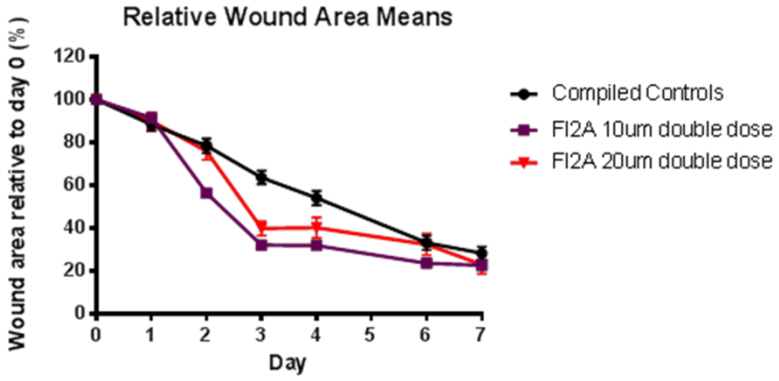


Figure 3: Enhanced wound closure following multiple dosing with Fidgetin-like 2 nanoparticle-siRNA

We have optimized treatment protocols using our original nanoparticle formulation. Starting next quarter, we will begin to test new nanoparticle formulations. One issue that needs to be addressed in relates to the solubility of our current nanoparticle forumlatio—nanoparticle precipitation is often observed and the extent of this can vary from experiment to experiment. This has complicated our dosing strategies. This will be addressed as described in Q1T2, above.

FY12 Quarter 1 Task 4. Start in vivo wound healing experiment
Subtask 4 a: All four targets, one concentration, single treatment at day zero.
This has been completed and, as expected, treatment of wounds with 10 μl of a 20μM solution of Fidgetin-like 2 nanoparticle-siRNA significantly enhanced wound closure relative to controls. We also found that treatment of wounds with either Kif19 or Cep192 nanoparticle-siRNA reduced the rate of wound closure suggesting potential efficacy as anti-fibrotic agents (Kif19 result shown in figure 2).

Subtask 4 b: Pick the winner from above (probably Fidgetin-like 2) and redo experiment using multiple applications
Task Q2 T5 (see also figure 9) was performed prior to Q1 4b in order to designate an appropriate nanoparticle concentration, before proceeding with multiple dosing of the nanoparticles. Once

this target concentration was determined (20um), an in vivo experiment was performed utilizing the previously described murine wound model. Groups were designated as follows, with five mice in each group: control mice (untreated), and two groups treated with double dosing of siRNA nanoparticles on Days 0 and 2. Samples were collected for histopathology and PCR, to be analyzed at a later date. From the sharp decline in relative wound area seen after the second does on Day 2, it is clear that fidgetin-like 2 knockdown accelerated wound healing. This sharp decline is not seen after the first dose on day 0 (figure 3). We hypothesize that due to inconsistencies in the nanoparticle suspension, adequate uniform concentration was not actually achieved in the day 0 dose. We plan to address this in the future by a PEGylated surface modification to the nanoparticle to improve its ability to remain in suspension.

FY12 Quarter 2 Task 1. Continue nanoparticle optimization (PEGylated derivatives and physical property characterization)
See Q1T2, above

FY12 Quarter 2 Task 2. Determine impacts of optimized np-si treatment protocols on microtubule organization and dynamics in U2OS and/or HekA cells.
This past quarter we have thoroughly documented the impacts of np-si mediated knockdowns of all four target proteins on the microtubule arrays of U2OS cells. Our findings include that:

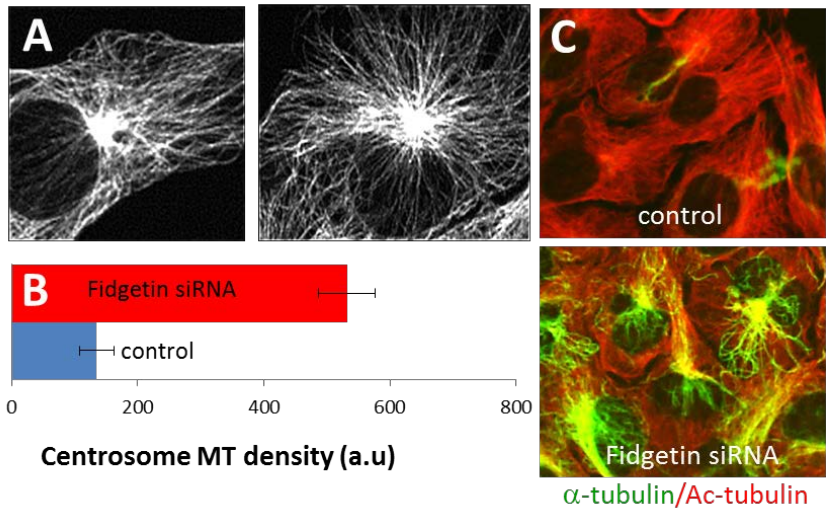


Figure 4: Fidgetin np-si treatment increases the density and stability of centrosomal microtubules in cultured cells. Panel A) high magnification immunofluorescence micrographs of control (left) and Fidgetin nanoparticle-siRNA treated (right) U2OS cells immunostained for alpha-tubulin (microtubules). The radial array of microtutubules is focused at the centrosome. Panel B) Quantification of the density of centrosome microtubules measured in each condition. Panel C) Double label immunofluorescence showing the increase in centrosome-associated acetylated microtubules (Ac-tubulin) after Fidgetin nanoparticle siRNA treatment.

<u>Fidgetin:</u> U2OS cells depleted of Fidgetin display a ~4-fold increase in the density of centrosome-associated microtubules which is accompanied by a significant increase in the accumulation of stable acetylated microtubules (Figure 4). Together, these data strongly support our working

hypothesis that Fidgetin normally severs and releases microtubules from their nucleation sites at centrosomes.

Fidgetin-like 2: Depletion of Fidgetin-like 2 from U2OS cells significantly increases the overall density of microtubules but decreases the density of stable acetylated microtubules (figure 5). Our current working hypothesis is that Fidgetin-like 2 selectively shears dynamic microtubules thus restraining the establishment of sustained cell polarity and directional motility. We have also found that

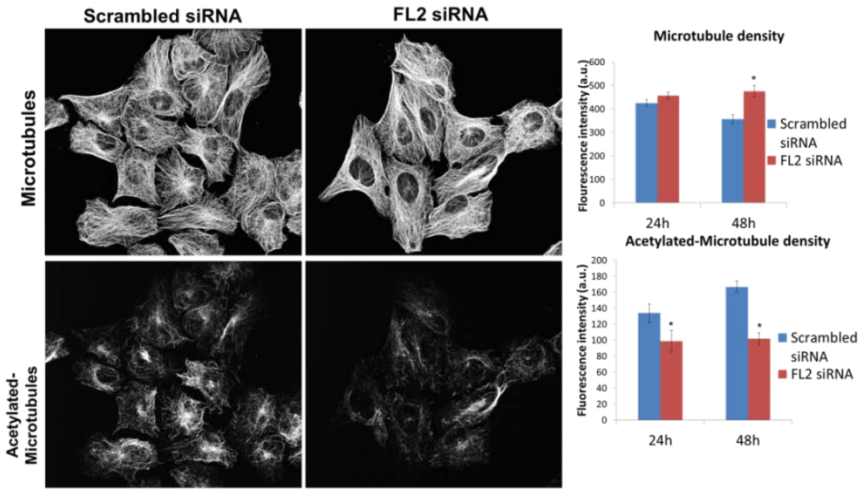
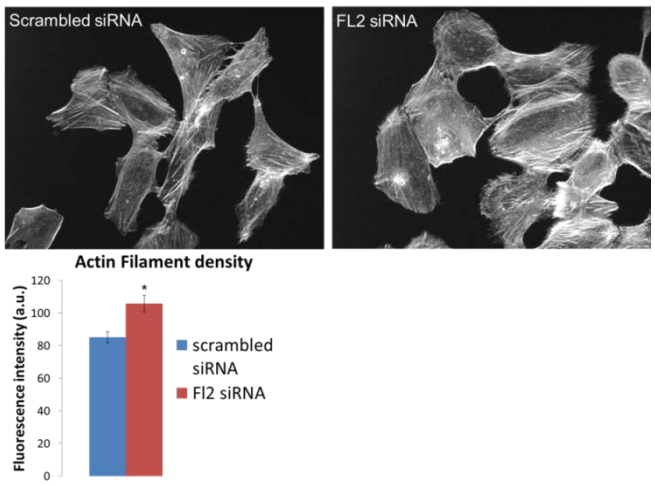


Figure 5: Depletion of Fidgetin-like 2 significantly alters microtubule organization and stability. (A) Immunofluorescence micrographs showing acetylated-microtubules and total microtubules in U2OS cells 48 hours after treatment with scrambled and FIGNL2 siRNA. (B) Fluorescence intensity quantification reveals an increase in microtubule density (top) and decrease in stable acetylated-microtubules (bottom) 48 hours after siRNA treatment. *P< 0.05. Mean values and S.E.M. are depicted as columns and vertical bars.



cells depleted of Fidgetin-like 2 contain increased F-actin levels (Figure 6). F-actin levels are known to be sensitive to alterations in microtubule dynamics. Alterations in actin organization and contractility indirectly caused by Fidgetin-like 2 siRNA induced shifts in microtubule dynamics could be an additional stimulatory factor driving the increased rates of cell motility in Fidgetin-like 2 np-si treated cells and tissues.

Figure 6: Fidgetin-like depletion results in an increase in actin filament density. (A) Immunofluorescence micrographs showing Phalloidin staining of actin filaments. (B) Quantification of the average Phalloidin fluorescence intensity as a measure of actin filament density. *P< 0.05. Mean values and S.E.M. are depicted as columns and vertical bars.

Cep192: U2OS cells depleted of Cep192 lose their radial centrosomal microtubule arrays, display an increase in the density of stable acetylated microtubules, and altered microtubule growth parameters (Figure 7). In addition, cells lacking Cep192 acquire hyperpolarized morphologies relative to controls. Finally, we have found that depletion of Cep192 from either U2OS cells or primary human keratinocytes (HekA) results in a strong and significant decrease in cell motility. These data are consistent with the hypothesis that Cep192 forms the scaffolding that recruits microtubule nucleating factors to the centrosome. In its absence, microtubules are nucleated from non-centrosomal sites (e.g. the Golgi apparatus). Golgi-derived microtubules are known to be highly acetylated and promote cell polarization.

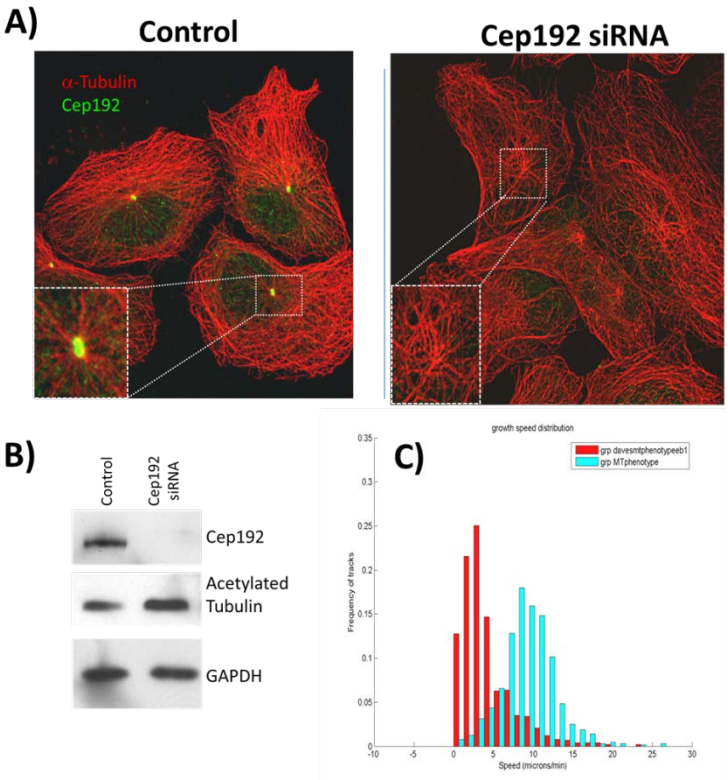


Figure 7: Depletion of Cep192 perturbs centrosomal microtubule arrays and microtubule growth. Panel A) U2OS cells treated with control or Cep192 nanoparticle siRNA were fixed and stained at 72 hours. Controls showed robust centrosomes with associatedc radial MT arrays while depleted cells were devoid of Cep192 staining and displayed non-radial MT arrangements. Panel B) Representative Western blot showing acetylated tubulin levels following Cep192KD. On average, acetylated tubulin increases ~64% following knockdown. GAPDH is shown as a loading control. Panel C) Histogram of microtubule plus-end growth

Kif19: Fluorescence Recovery After Photobleaching analysis of of GFP-labeled focal adhesions in control and Kif19 np-si treated cells has incidated that Kif19 normally controls adhesion size and stability by regulating the dynamic turnover of proteins within the adhesion itself (not shown).

FY12 Quarter 2 Task 3. Determine impacts of optimized np-si treatment protocols on rates of axon outgrowth.

In the past quarter, we have analyzed the impact of np-si treatment on rates of central nervous system axon outgrowth (Figure 8). We generated cultures of E18 rat hippocampal neurons, plated at 4000 cells/dish. Cells treated with either Fidgetin or Fidgetin-like 2 nanoparticle siRNA showed a significant increase in rate of axonal outgrowth in comparison to controls (Students t-test, $p<0.05$). Please note that our previous results suggested that only Fidgetin affected the rates of axon outgrowth. Our working hypothesis has been revised accordingly. Indeed, this latest result is consistent with parallel in vivo spinal cord injury studies showing that Fidgetin-like 2

nanoparticle siRNA treatment of crushed spinal columns in rats significantly improves locomotion and bladder function within a matter of days (see Addendum).

FY12 Quarter 2 Task 4. Optimize np-si treatments in embryonic hearts: Determine knockdown efficiency using QPCR.

We have been able to achieve a ~80% decrease in target mRNA levels by culturing embryonic hearts in matrigel containing a final concentration of 200 nM np-si (not shown).

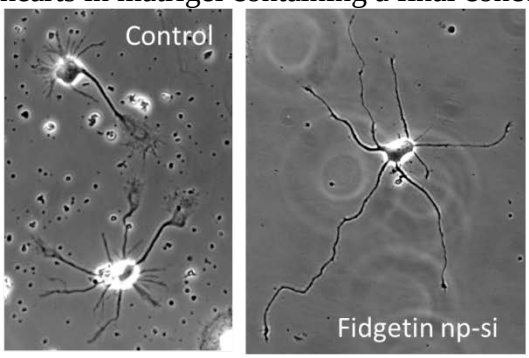
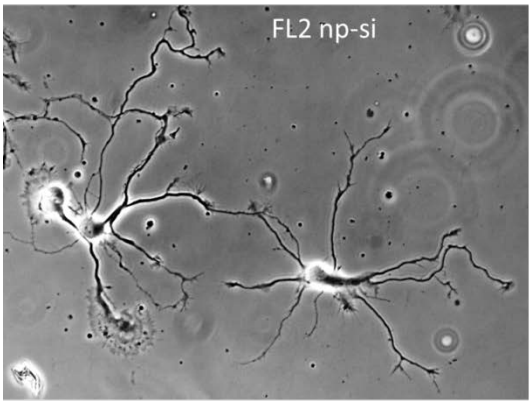
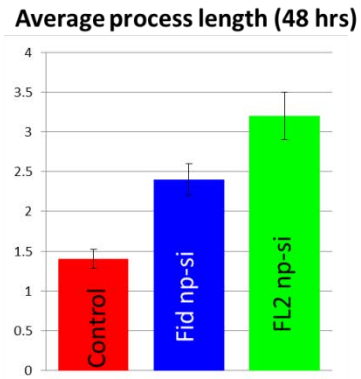


Figure 8: Fidgetin and Fidgetin-like 2 nanoparticle-encapsulated siRNAs strongly promote axon regrowth from hippocampal neurons

Images show dissociated rat hippocampal neurons 48 hours after nanoparticle-siRNA treatment.





FY12 Quarter 2 Task 5. In vivo wound healing assay continued.

Subtask 5a: Dose Response

This task (Q2T5a) was performed prior to Q1T4b in order to designate an appropriate concentration before continuing with multiple dosing. After investigating four gene targets, it was determined that fidgetin-like 2 was the most promising in terms of wound healing. Once this gene target was selected, it was necessary to determine the most effective concentration of nanoparticles to be applied to the wounds. An in vivo experiment was done utilizing the previously described murine wound model. Five groups were designated, with five mice in each group: control mice (untreated), and four groups treated with varying concentrations of fidgetin-like 2 siRNA nanoparticles. Wounds were serially measured and photographed for 10 days. Samples were collected for histopathology and PCR. The results shown in figure 9 demonstrate that 20um is likely the most accurate concentrations for achieving knockdown without toxic effects. It has been demonstrated in the literature that excess amounts of siRNA can overwhelm the endogenous cellular machinery, disrupt vital cell processes, and lead to toxic effects.

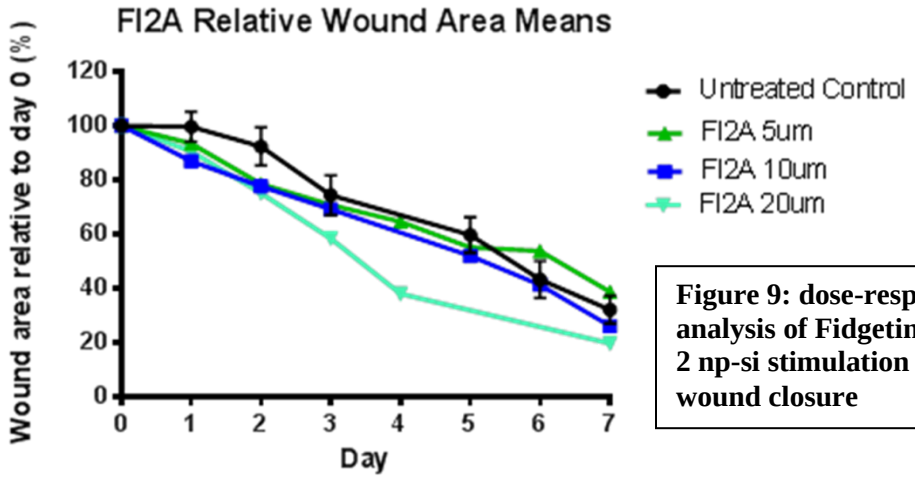


Figure 9: dose-response analysis of Fidgetin-like 2 np-si stimulation of wound closure

Subtask 5b: Comparison with Regranex: This task was not performed as our supplier no longer carries Regranex.

FY12 Quarter 3 Task 1. Continue nanoparticle optimization

One issue that has confronted us with respect to being able to deliver a precise amount of the siRNA loaded nanoparticle is the excipient to be used as a carrier for the particles. Sample inhomogeneity has been a limiting factor. We have developed a coconut oil based delivery vehicle that allows for: i) uniform mixing of the nanoparticles into the coconut oil; ii) preparation of a fine powder version of the coconut oil plus nanoparticles that melts on contact with living tissue; iii) preparation of a solid "stick" form of the coconut oil plus nanoparticles that can be used in a manner analogous to chap lip or lipstick; and iv) design of patches that can act as a dressing for wounds.

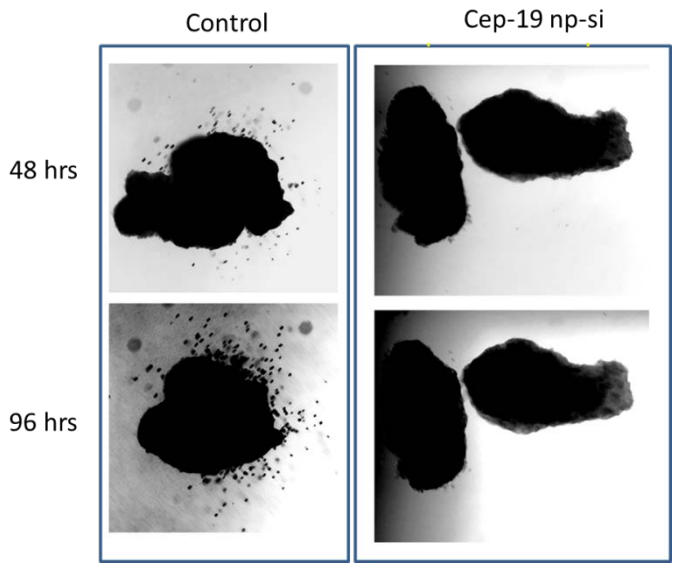


Figure 10: Inhibition of cell motility from anaplastic thyroid carcinomas (mouse) embedded in Matrigel. Images show control and Cep192 np-siRNA treated tumors at 48 and 96 hours in matrigel

FY12 Quarter 3 Task 2. Optimize imaging and treatment conditions for in vitro wound healing assays

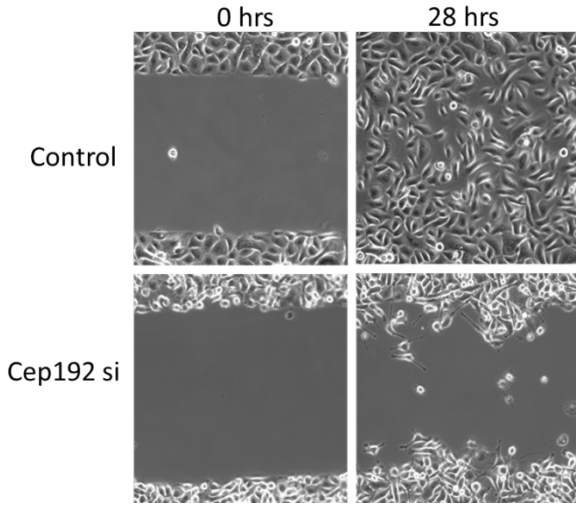
We have developed 3-D migration assays in which tumors are dissected from mice and embedded in matrigel containing nanoparticle siRNAs. As shown in figure 10, cells from control tumors are easy to observe as they invade the matrigel and migrate away from the primary tumor. By contrast, very little cell movement from the primary tumor is observed when tumors are embedded in matrigel containing Cep192 or Kif19 np-si. (Figure 10)

In addition to these 3-D assays, we have also optimized conditions for 2-D imaging of primary human adult keratinocyte motility. siRNA depletion of Cep192 strongly inhibits the movement of these cells (Figure 11; next page)

FY12 Quarter 3 Task 3. Ex vivo angiogenesis quantification with optimized np-si treatment

This task was been initiated and completed ahead of schedule. We have found that treatment of embryonic hearts with either Fidgetin-like 2 or Fidgetin np-si significantly increases new vessel formation. Alternatively, Cep192 np-si strongly inhibits vessel formation. These experiments have been repeated several times and the results are highly significant

In addition, we have carried out a pilot study examining whether Fidgetin-like 2 np-si improves cardiac output after infarct in vivo. In this study, infarction was induced in mice by permanently ligating the left descending coronary artery. Control or Fidgetin-like 2 np-si was applied topically to the surface of the heart immediately after coronary artery ligation. Aortal output was examined weekly in control and Fidgetin-like 2 np-si treated mice by echocardiograms. We measured a strongly significant ~40% increase in aortal output by 4 weeks after infarction in Fidgetin-like 2 np-si treated animals relative to controls (Figure 24; 10 animals were measured in each condition). Follow-up histological analysis also revealed a strong decrease in infarct size after fidgetin-like 2 np-si treatment (figure 25).



FY12 Quarter 3 Task 4. Characterization of microtubule dynamics and organization in np-si treated adult DRG neurons

Using adult rat DRGs, we have completed the nanoparticle studies for fidgetin and fidgetin-like 2 on axon extension, and axon growth on inhibitory substrates (figure 12). We have found that either Fidgetin or Fidgetin-like 2 promotes axon growth through aggrecan (CSPG) stripes in vitro. Aggrecan stripes are a widely used in vitro model for an inhibitory glial scar. These data help to explain the data shown in the addendum indicating that fidgetin-like 2 np-si treatment promotes functional recovery after spinal cord injury in vivo.

Figure 11: In vitro wound healing assay using primary adult ketatinocytes

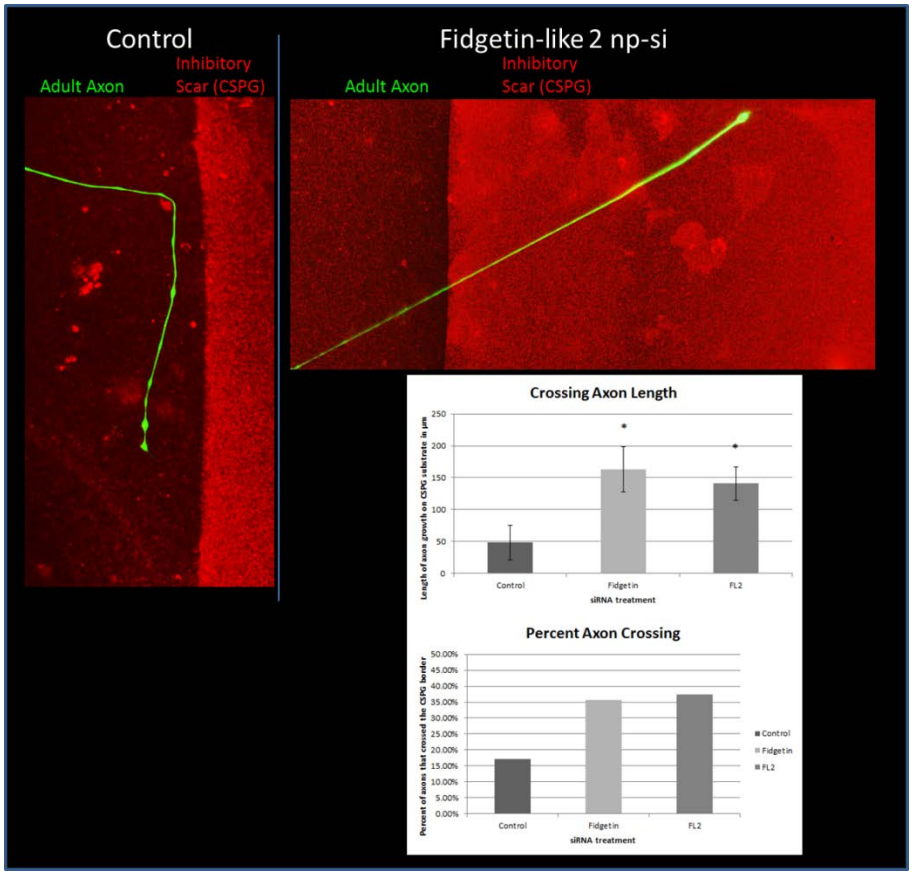


Figure 12: Fidgetin and Fidgetin-like 2 np-si promote the growth of adult DRG axons through inhibitory substrates in vitro (CSPG model of glial scar)

In addition, in order to further characterize the impacts of fidgetin np-si on microtubule dynamics, we used 2 µg/mL of Nocodazole to inhibit microtubule polymerization across different time points in adult DRG neuronal cultures exposed to either siFidgetin or control nanoparticles (2 µl/mL). Nocodazole binds to free tubulin and prevents it from polymerizing with dynamic microtubules. Similar to what was previously reported by Baas & Black (1990), microtubule fluorescence rapidly decreased by half when neurons were treated with Nocodazole for only eight minutes (Figure 13). After two hours, microtubule fluorescence continued to gradually decrease a total of 25 percent (Figure 13). This biphasic effect is indicative of different microtubule domains. A dynamic domain of microtubule is extremely sensitive to Nocodazole and makes up the first phase of lost fluorescence. The second phase of microtubule mass that slowly decreases over time is resistant to Nocodazole treatment and thus does not undergo rapid exchanges between microtubule-bound and free tubulin in the same manner as the dynamic form of microtubule. Stable domains are considered to be highly post-translationally modified, some of which are stabilizing modifications. There was no difference between adult DRG neurons treated with either siFidgetin or control nanoparticles (Figure 13). This is counter to what is seen with taxol, which grants microtubules the ability to resist Nocodazole treatment, but in a manner that dramatically alters their dynamic-to-stable ratio.

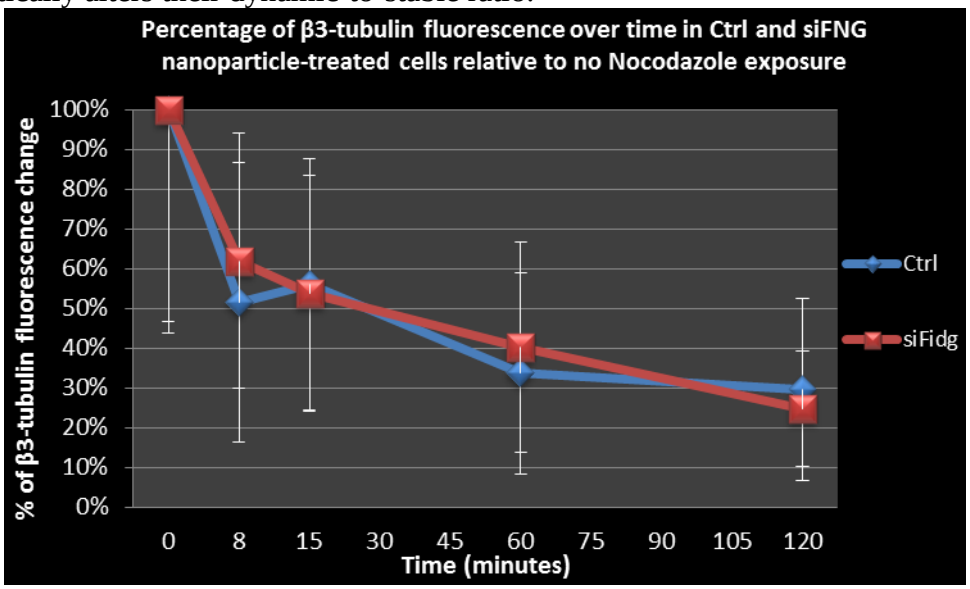


Figure 13. The Percentage of β3-tubulin fluorescence over-time in control and siFidgetin nanoparticle-treated cells, relative to neurons not treated with Nocodazole. Adult DRG neurons treated with either control or siFNG nanoparticles were exposed to the same concentration of nocodazole over different periods of time and show no differences in the biphasic nature of lost fluorescence relative to the fluorescence intensity of neurites not treated with Nocodazole. Individual neurites were imaged at the same exposure across all dishes at 100xoil magnification. Within the program Image the images were converted to 8-bit, neurites were individually outlined, and the mean grayscale was measured. To obtain the percentage, each condition's mean greyscale value was divided by the mean grayscale value of its corresponding nanoparticle treated dish that was not exposed to Nocodazole. The decrease in β3 tubulin fluorescence overtime as a result of Nocodazole exposure is biphasic. Within the first 8 minutes of Nocodazole exposure microtubules decrease in fluorescence intensity by half and continue to decrease between 8 and 120 minutes by approximately 25%. This indicates dynamic (first phase) and stable (second phase) portions of microtubule.

However, there was an increase in the amount of overall β3 tubulin in cultures of siFidgetin treated adult DRG neurons. We found, both qualitatively (Figure 14 A & B) and quantitatively (Figure 15 A), that adult DRG neurons treated with siFidgetin nanoparticles resulted in a greater amount of microtubule outgrowth as well as increased β3 tubulin area coverage. The data also indicates that following siFidgetin nanoparticle treatment there may be more total neurons staining positive for β3 tubulin (Figure 15 B), as well as a higher percentage of cells extending neurites (Figure 15 C).

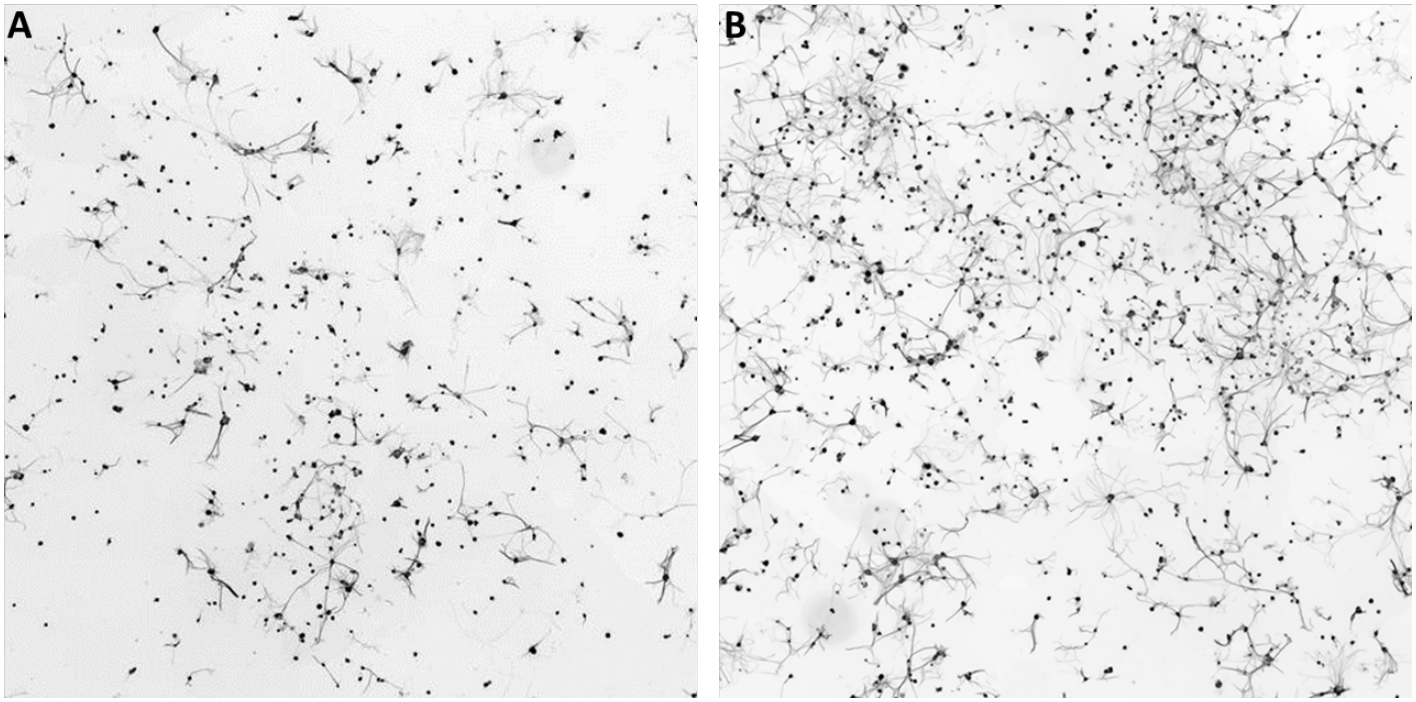


Figure 14. Adult DRG neurons with β3 tubulin fluorescently labeled following 2 days in culture with media containing control (A) or siFNG (B) nanoparticles. After staining for β3 tubulin, culture dishes containing adult DRG neurons treated with nanoparticles were imaged under a fluorescence microscope at 10x magnification. Images were captured spanning the entire dish and stitched together in Microsoft ICE, producing large and highly detailed panoramic images. There is a greater amount of neurite outgrowth as well as cells growing neurites in siFGN nanoparticle treated neurons (B) compared to control nanoparticle treated neurons (A).

Taken together, our results indicate that the microtubule density is increasing in adult DRG neurons following treatment with siFidgetin nanoparticles, yet the ratio of dynamic to stable microtubule domains is unaffected. These results are promising considering a single treatment with siFidgetin nanoparticles is capable of improving the *in vitro* regenerative capability of adult DRG neurons after being excised from living tissue without dramatically altering the intrinsic dynamic-to-stable ratio of neuronal microtubules. Compare this to taxol, which alters microtubule dynamics in a manner that increases the amount of stable to labile ratio and makes axons rigid battering-rams. It would seem that siFidgetin treated adult DRG neurons exhibit a normal morphology with normal microtubule dynamics, simply there are *more* neurites in total. Replications of this work are required to reveal statistically-significant findings. Currently we are working to co-immunostain for β3 tubulin and neurofilamets so as to compare

the ratio of β3-tubulin to neurofilaments. This ratio will reveal whether the increase in microtubule mass we observed is proportional to the neurite as a whole, considering neurofilaments are more abundant in neurons than microtubules and assumingly unaffected by microtubule severing proteins. Furthermore, the success of Fidgetin-like 2 in other studies requires for these same experiments to be reproduced using siFNG-L2 nanoparticles.

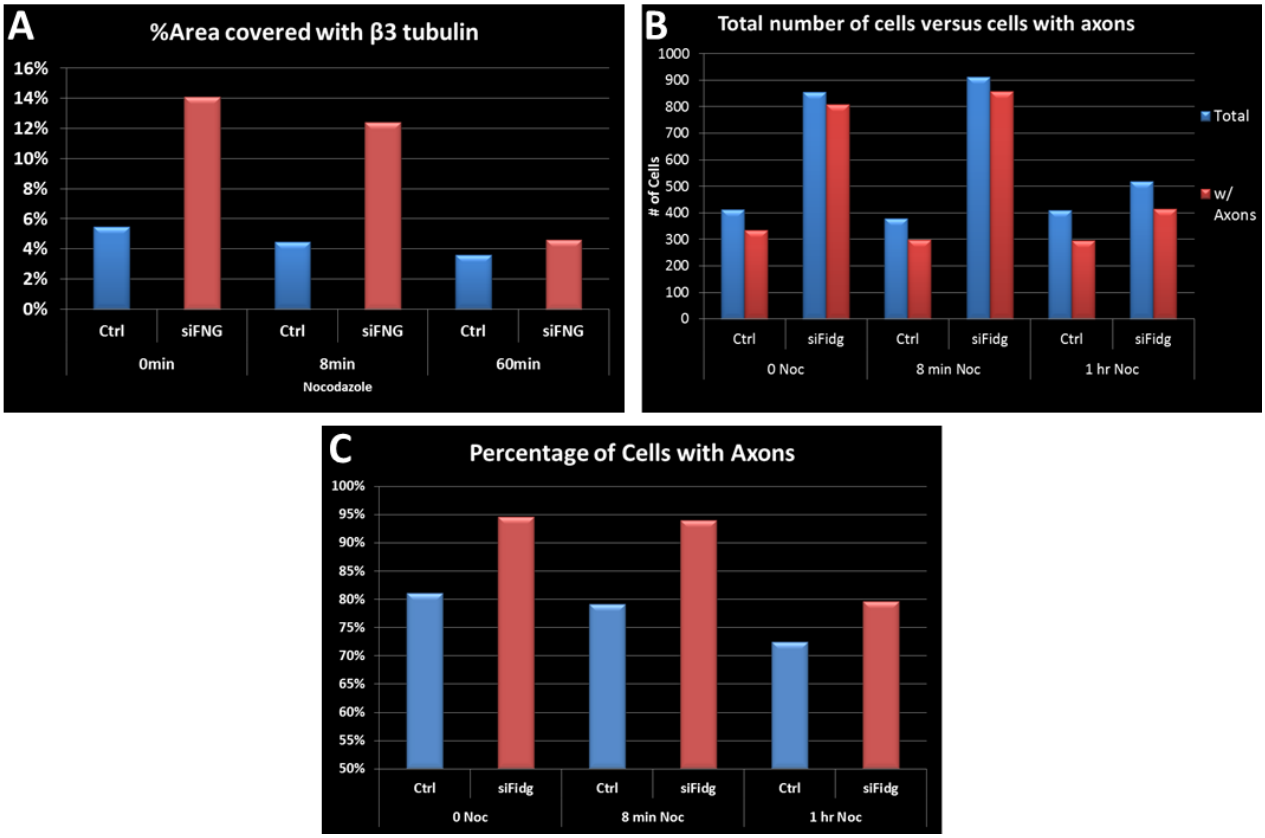


Figure 15. Quantitative analysis of panoramic 10x images stitched together by Microsoft ICE and analyzed using ImageJ. The area of the dish covered by β3-tubulin-labeled cy3-fluorescence was measured in ImageJ.A) The percentage of area in a 5000 by 5000 pixel square was measured and adult DRG neurons treated with siFidgetin had approximately 10% more area coverage compared to controls. B) The total number of neurons with and without neurites was greater in cells treated with siFidgetin nanoparticles and C) the percentage of cells with neurites was higher in neurons treated with siFidgetin nanoparticles.

FY12 Quarter 3 Task 5. Start work in vivo burn model using the optimized protocols determined from the above rounds of wound healing studies.

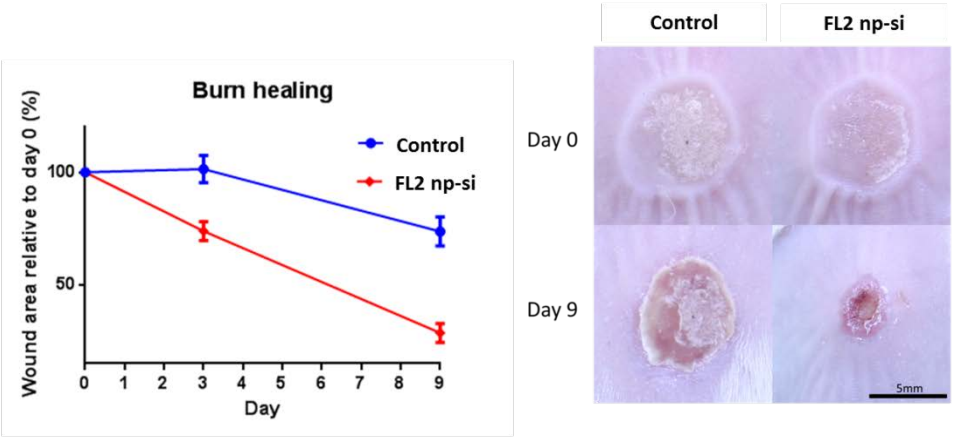
This task has been completed. We have found that topical application of 10 micromolar Fidgetin-like 2 np-si significantly increases the rate of burn healing relative to controls (figures 16 & 17). Higher concentrations of Fidgetin-like 2 np-si also promoted burn healing but to a lesser extent (not shown). Treatment of burns with 5 micromolar Fidgetin-like 2 np-si was not an effective pro-healing agent (not shown).

In addition to the burn healing studies, we have completed a complete histological analysis of our excision wound healing results. These data indicate that Fidgetin-like 2 np-si not only

promotes wound closure, but actually stimulates complete regeneration. The histological images shown in figure 18 indicate that wounds treated by Fidgetin-like 2 np-si display normal morphological features such as hair follicles much earlier than control wounds.

Figure 16

Topical application of Fl2 np-si promotes burn healing



Reproducible burn wounds are created on the flanks of anesthetized, debilitated mice. Wounding occurs by applying heated 5 mm brass bars to a flank for 45 s. Left) Graph shows average relative wound area s measured from control or FL2 np-sitreated burns (topically administered on days 0, 2, 4, and 8 after wounding). Right) images showing representative control and enhancer-treated burn wounds at 0 and 9 after wounding

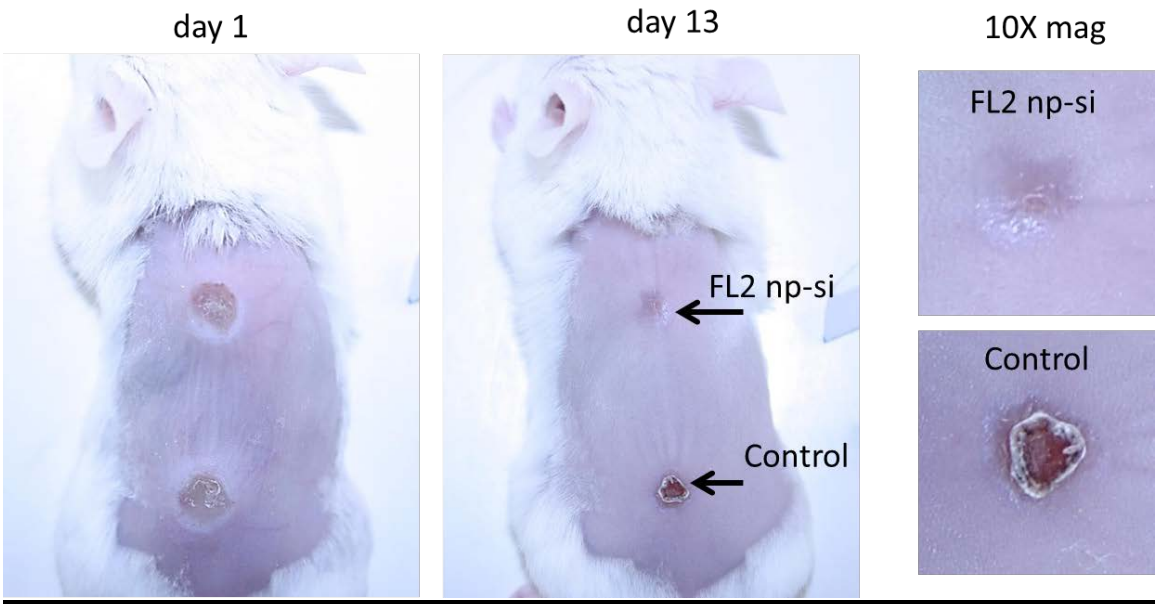


Figure 17: Mouse with two burns on its flank. The top wound was treated with FL2 np-si while the bottom wound was treated with control np. By day 13, the burn treated with FL2 np-si has closed while the control treated wound remains open.

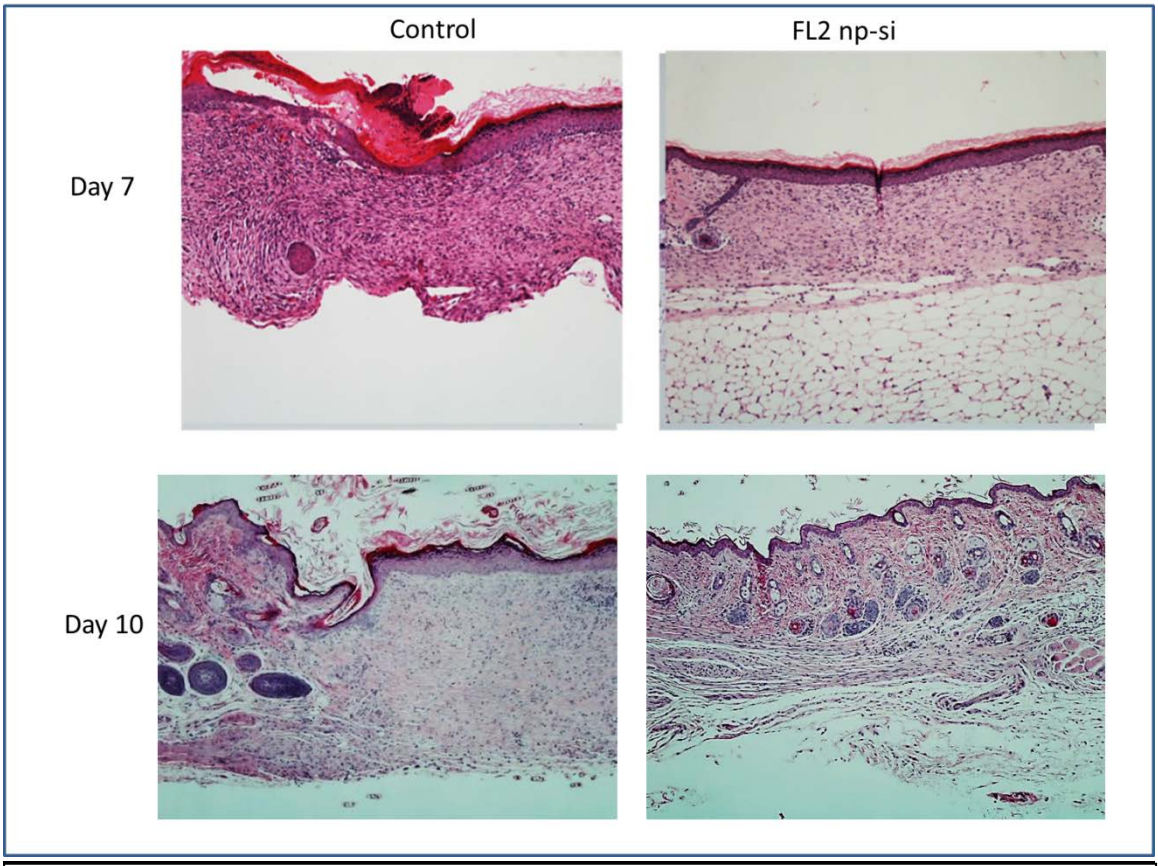


Figure 18: Representative H&E stained sections of control and fidgetin-like 2 np-si treated mouse excision wounds at days 7 and 10 after wounding. Please note that by day 10, important structural features such as hair follicles have been restored in the fidgetin-like 2 np-si treated wound but not in the control.

FY12 Quarter 4 Task 1.Complete screening of optimized nanoparticles using in vitro wound healing, neuron regeneration, and ex vivo coronary angiogenesis models
This task was completed ahead of schedule (during Q3)

FY12 Quarter 4 Task 2. Generate usable quantities of quantities of optimized nanoparticles
This task has been completed as proposed

FY12 Quarter 4 Task 3. Test efficacy of optimized nanoparticles in the in vivo excision and burn models
This task has been completed as proposed

FY12 Quarter 4 Task. Prepare and submit data for publication
Our studies showing that Fidgetin-like 2 promotes cutaneous wound healing will be submitted to Science Translational Medicine by the end of November. We will also be submitting a manuscript on Cep192 to PLOS*One* in the next few weeks. Finally, an abstract of our findings showing that Fidgetin can be targeted to promote neural regeneration has been chosen for a press release at the American Society for Cell Biology Annual Meeting held in New Orleans from December 13-18.

Addendum: Additional data related to but not directly supported by the current grant

In addition to the above, we have conducted 2 pilot studies relevant to the therapeutic potential of our technology.

Pilot study 1: Functional recovery after spinal cord injury induced by topical application of Fidgetin-like 2 siRNA nanoparticles.

We have found that a single local application of our Fidgetin-like 2 nanoparticle-siRNAs to crushed spinal cord regions (dura intact) in rats significantly enhances motor function and bladder control within 4-5 days. Histological analysis (H&E staining) also indicates enhanced tissue structure and reduced lesion size after Fidgetin-like 2 siRNA nanoparticle treatment.

Methods:

Eight adult female Wistar rats (250-280g body weight, Charles River Laboratories, Inc.) were used for this pilot study. Immediately after a moderate traumatic spinal cord injury (SCI), rats were randomly divided into two groups. Animals in one group received 40µl of 20µM Fidgetin like-2 (Fl-2) nanoparticle-siRNA locally at the injury site (with dura intact) while animals in a second group received the same volume of vehicle control (RNase free water). Fl-2 nanoparticles are in lyophilized form containing 30 nmols of siRNA. We suspended them in 1.5 ml of RNase free water to make a 20uM solution and store aliquots in the -80.

Motor functional recovery assessment

All rats were handled daily for two weeks preoperatively to accustom them to the handling and behavioral testing. After injury, the locomotor behavior of the rats was assessed daily using a standard open field walking test. The open-field walking test was conducted in a circular empty plastic swimming pool. They were observed for 5 min periods and scored for general locomotor ability using the standard Basso-Beattie-Bresnahan locomotor rating scale. The rats were rated on a scale of 0 to 21, with a value of 0 corresponding to complete loss of function and 21 to normal function. If the animal stopped moving for a minute, it was placed again in the center of the open field; otherwise, it was left undisturbed for the duration of the 5 min test period. Behavioral assessment was performed by 3 individuals who were blinded to the treatment (Fl-2 or vehicle). At 7 days after injury, all animals were sacrificed for histological analysis.

Recovery of lower urinary tract function

Normal lower urinary tract (LUT) function involves both spinal and supraspinal circuitry that controls urine storage and release. Incomplete SCI results in an initial loss and later partial or complete recovery of LUT function depending on the severity of injury. After SCI the rats were not capable initially of spontaneous micturition, and their bladders were manually expressed twice daily. The volume of expressed urine was measured each time, and the data were used to estimate the initiation of LUT function after SCI.

Results:

1. Local application of Fl-2 nanoparticle-siRNA to the injured spinal cord improves locomotor functional recovery following an acute SCI

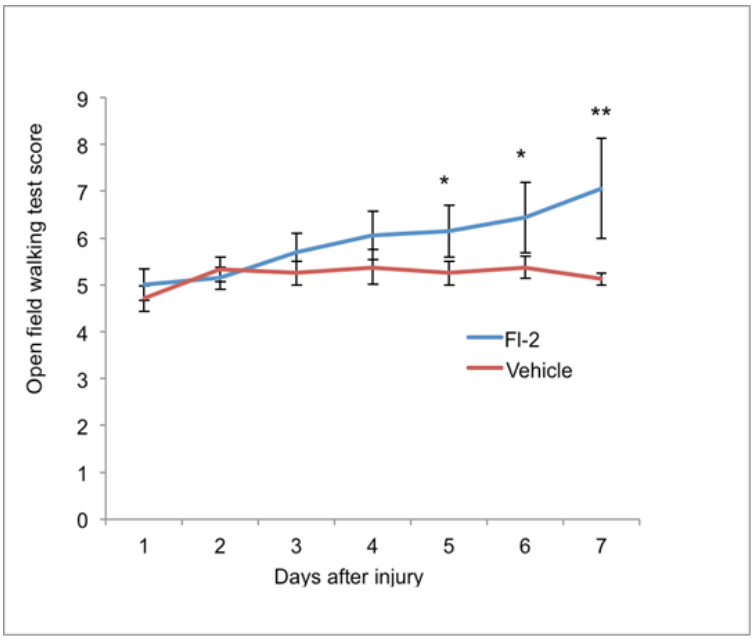


Figure 19: Open field walking scores from day 0 (the day of surgery) to day 7 after spinal cord injury for groups of vehicle control- and Fl-2-nanoparticle siRNA-treated animals (means ± SEM). Animals with normal spinal cord function scored 21, whereas a score of 0 represents total paralysis. Hindlimb locomotor function in Fl-2 nanoparticle-siRNA-treated animals recovered significantly better than in control rats from day 5 to 7 after injury (*$p < 0.05$, **$p < 0.01$; Student T-test; n = 3 for vehicle control group since one rat died at day 2 because of bladder infection from this group; n=4 for Fl-2 group).

2. Local application of Fl-2 nanoparticle-siRNA to the injured spinal cord accelerates LUT functional recovery following an acute SCI

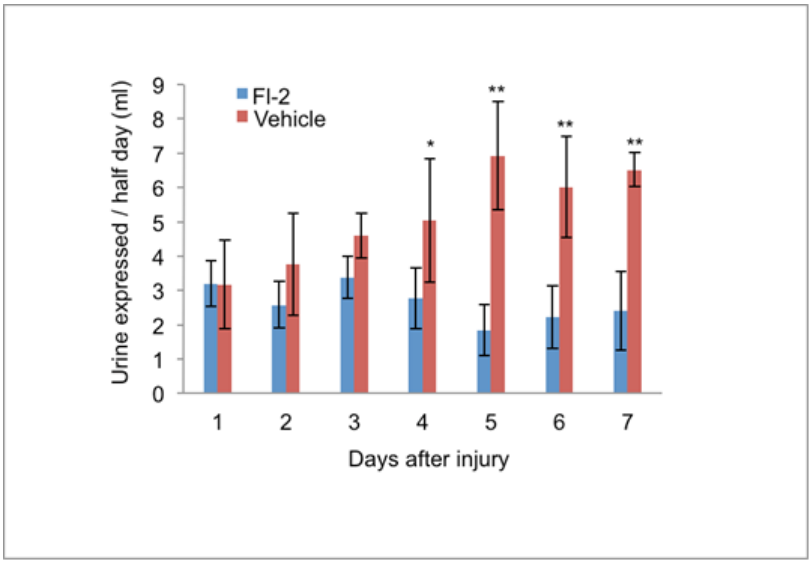
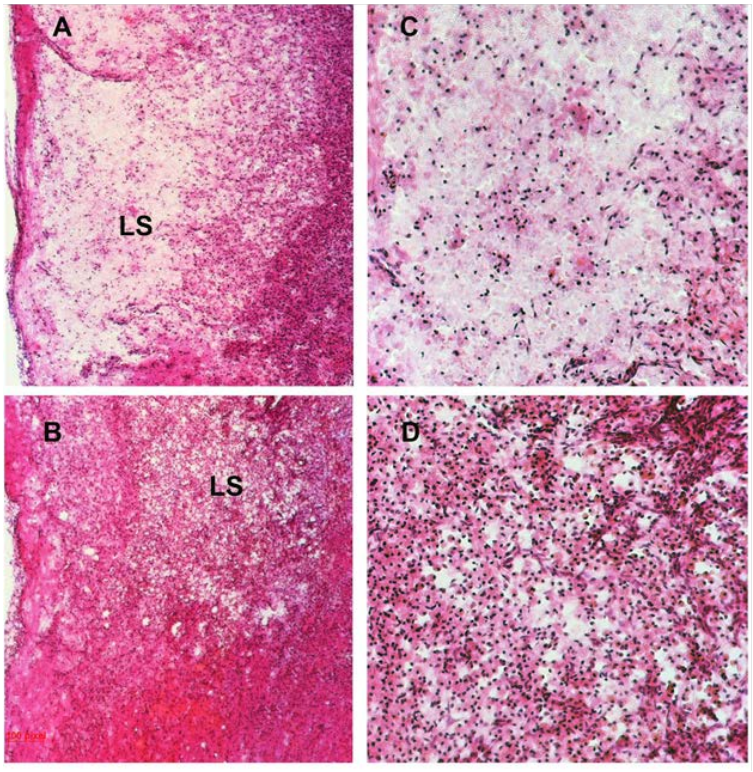


Figure 20. Time course of recovery of lower urinary tract function (spontaneous voiding). Urinary bladders were expressed every 12 h, and the collected urine volume was measured. Means ± SEM of the volume for each group. Compared with vehicle-treated control animals (n=3), Fl-2 nanoparticle-siRNA-treated animals (n=4) had significantly less urine collected with time from day 4 onward after SCI (*P<0.05; **P<0.001; Student T-test).



3. Local application of Fl-2 np-siRNA enhances tissue structure after acute SCI

Figure 21. Representative photomicrographs of longitudinal thoracic spinal cord sections at the injury site stained with hematoxylin and eosin (H&E) 7 days post-injury from (A) vehicle control-treated animals; (B) Fl-2 nanoparticle-siRNA

Pilot study 2: Promotion of new vasculature and vascular remodeling in infarcted adult hearts after topical application of Fidgetin-like 2 siRNA nanoparticles.

We have found that topical application of Fidgetin-like 2 nanoparticle siRNAs promotes angiogenesis, reduces infarct size from adult hearts in a murine infarct model.

Methods:

Following intubation and thoracotomy, Fidgetin-like 2 nanoparticle-siRNA or nanoparticles encapsulating scrambled RNA (control) was applied to the anterior surface of hearts in situ of intact 8-10 week old male C57Bl/6 mice. Following this, the proximal left coronary artery was completely occluded with a suture inducing marked pallor, indicative of ischemia, in the anterior wall of the heart. Animals were sacrificed 7 d later, following which hearts were excised, perfusion fixed, frozen, cryosectioned and immunostained for PECAM1, a marker of vessel endothelial cells, and smMHC, a marker for smooth muscle cells.

Results:

Microscopic examination showed marked angiogenesis in hearts treated with Fidgetin-like 2 nanoparticle siRNA, but not control siRNA. In animals receiving Fidgetin-like 2 siRNA, angiogenesis was limited to the treated anterior wall as indicated by PECAM1 staining (figure 14) and not seen in the untreated posterior wall. These findings were confirmed by immustaing for smMHC (figure 15), a second marker of angiogenesis. We conclude that knockdown of Fidgetin-2 like induces marked angiogenesis in the adult murine heart. These results suggest that Fidgetin-like 2 nanoparticle-siRNA may provide a novel therapeutic approach to human ischemic syndromes, including myocardial infarction, stroke, disease, and traumatic injury to the extremities.

Figure 22

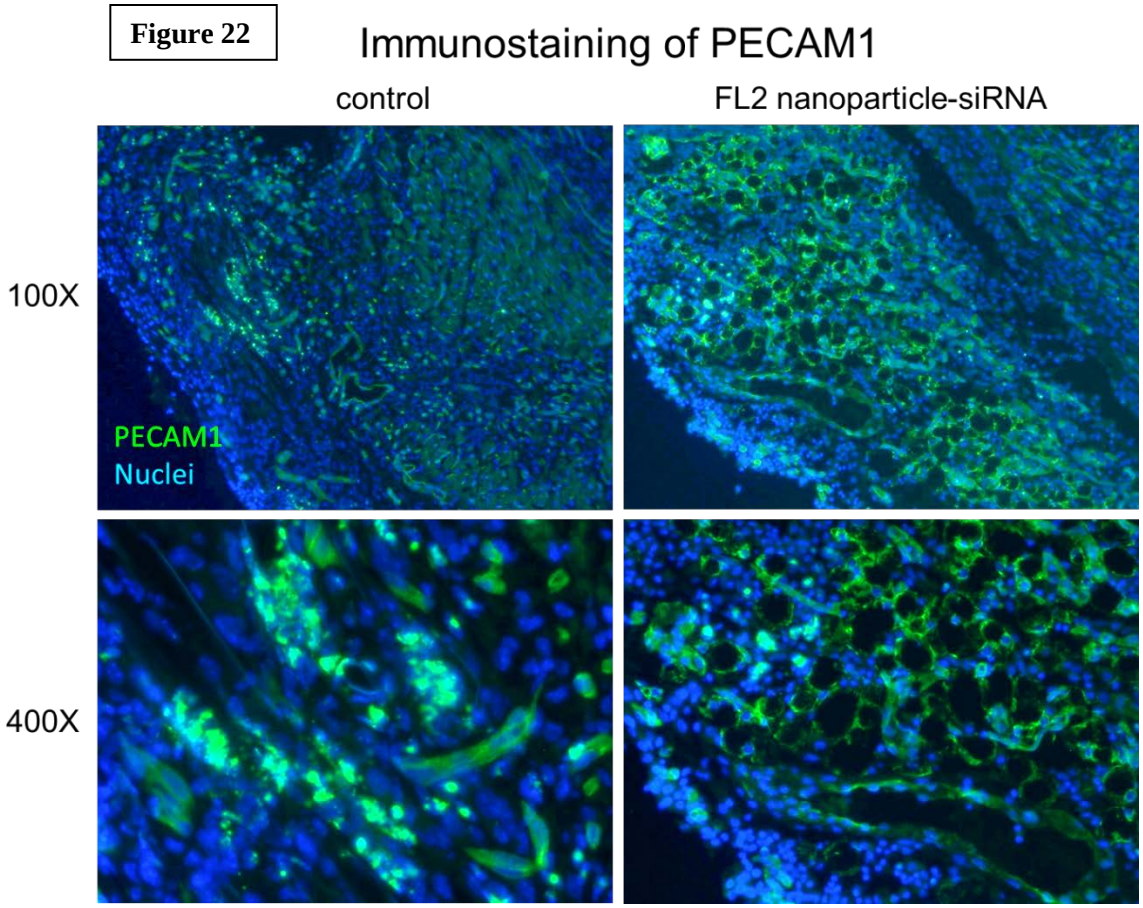

Immunostaining of PECAM1
control
FL2 nanoparticle-siRNA
100X
400X
PECAM1
Nuclei

Figure 23

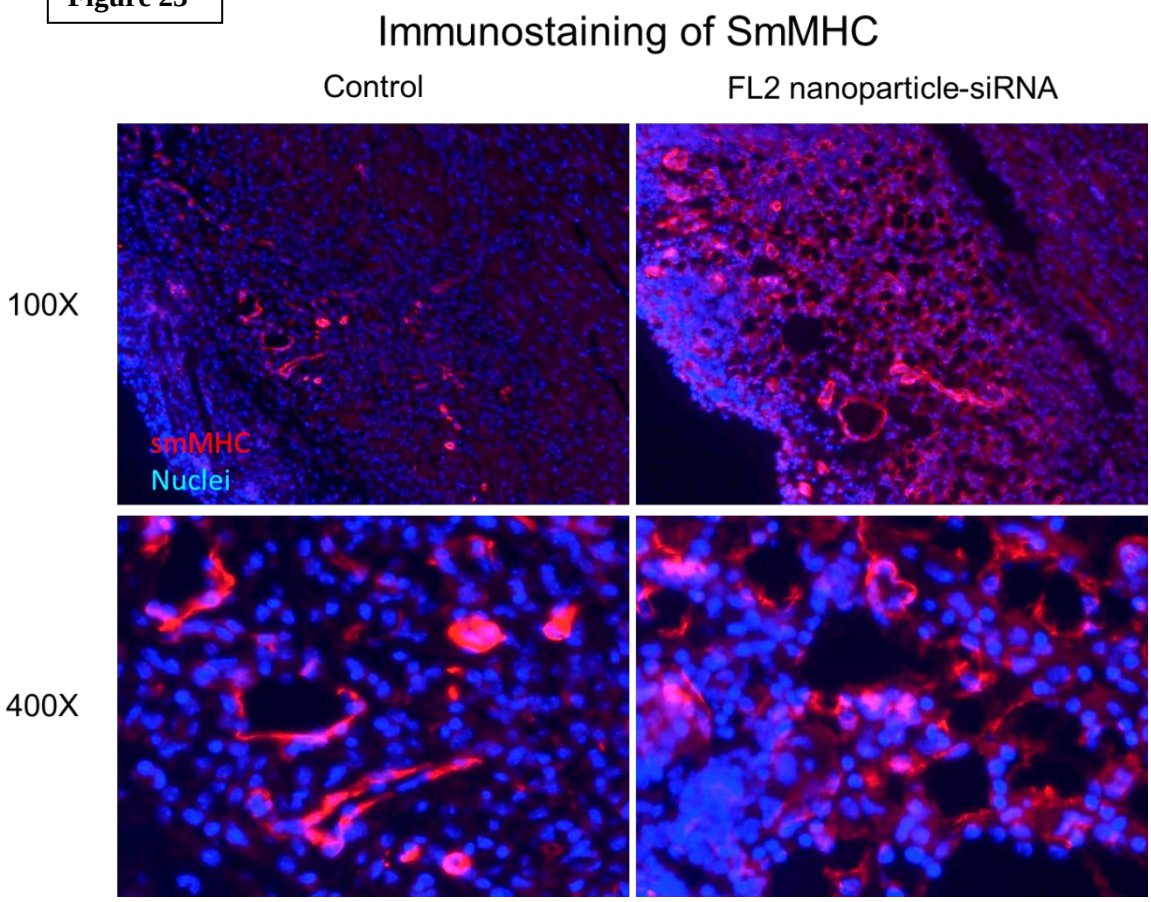
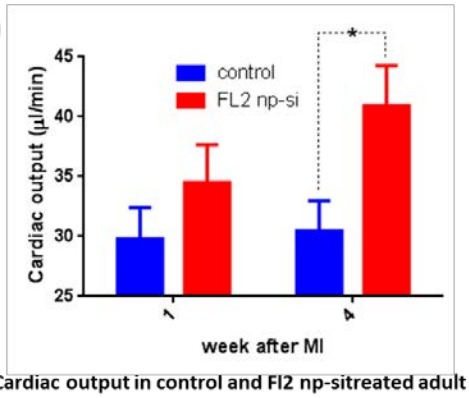

Immunostaining of SmMHC
Control
FL2 nanoparticle-siRNA
100X
400X
smMHC
Nuclei

Figure 24

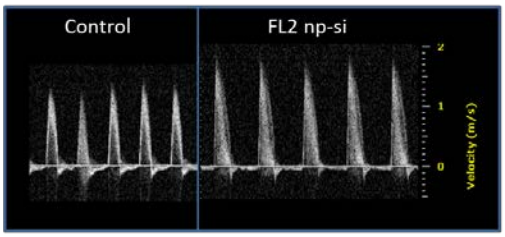
FL2 np-si treatment improves gross cardiac output after MI

A)



Cardiac output in control and Fl2 np-sitreated adult mouse hearts 1 and 4 weeks after myocardial infarction was induced by a permanent ligation of the left ascending coronary artery. Cardiac output was measured as the product of aortal stroke volume (determined by echocardiogram; see panel B) with heart rate. n=8, 10 mice, respectively. *FL2 np-si treatment resulted in a significant improvement in cardiac output by 4 weeks; p=0.0055

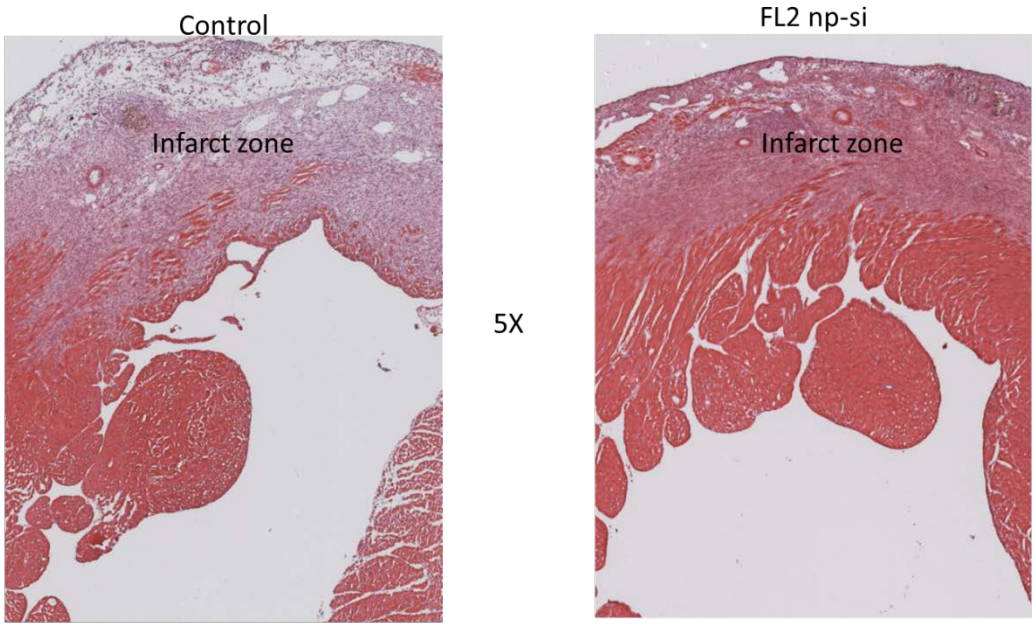
B)



Representative doppler wave forms from echocardiograms measuring aortal stroke volume in control and FL2 np-si treated hearts. Measurements shown here were made 4 weeks after myocardial infarction.

Figure 25

Histology indicates that application of FL2 np-si to infarcted hearts reduces ischemia and promotes regeneration of the myocardium and vasculature



4. KEY RESEARCH ACCOMPLISHMENTS:

a. Cell Biology

i. We have shown that Fidgetin-like 2 is an important regulator of microtubule organization and dynamics in a variety of cell types. Our data also suggest that it functions specifically by shearing microtubules at the leading edge of motile cells

ii. We have shown that Fidgetin is an important regulator of microtubule interactions with centrosomes

iii. We have shown that Cep192 is required for the nucleation of centrosomal microtubules

iv. We have shown that Kif19 regulates microtubule dynamics at focal adhesions

b. Therapeutics

i. We have shown that topical application o Fidgetin-like 2 nanoparticle encapsulated siRNA promotes the regeneration of cutaneous excision wounds and burns and have identified optimal conditions for use.

ii. We have shown that Fidgetin and Fidgetin-like 2 nanoparticle encapsulated siRNA promotes regeneration of neurons from either the central or peripheral nervous systems.

iii. We have also found that Fidgetin or Fidgetin-like 2 nanoparticle siRNAs promote neuron growth through inhibitory substrates present within glial scars which normally prevent functional recovery after spinal cord injury. In a related study, we have shown that treatment of crushed

spinal columns in rats with Fidgetin-like 2 nanoparticle-encapsulated siRNA significantly enhances locomotion and bladder function

iv. We have found that Fidgetin-like 2 nanoparticle siRNAs strongly promotes new blood vessel formation. This approach has been used in vivo to reduce ischemic damage and increase cardiac output after myocardial infarction.

v. We have shown that Cep192 or Kif19 nanoparticle encapsulated siRNAs inhibit cell migration using physiologic 3-D migration assays—this assay specifically monitored the movement of tumor cells away from a primary tumor embedded in matrigel.

vi. We have shown that Kif19 can be targeted to slow the movement of cells into wounds in vivo and thus potentially provides a means to inhibit scarring.

CONCLUSION: The completion of this study has accelerated the development of a first generation of topically delivered therapeutics that can be tailored to address the wide range of injuries incurred in even the most difficult military situations. This technology utilizes an entirely new mode of action, optimizing cell motility by altering the expression of protein regulators of the microtubule cytoskeleton. The resulting therapeutics will be easy to deploy in the field by either soldiers or medics allowing for immediate augmentation of the wound healing process. Enhanced wound closure will decrease the risk of infection or other complications associated with open injuries and will also translate into reduced recovery time that will speed the return of injured troops to active duty or productive civilian life.

5. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

(1) Lay Press: Our work on neural regeneration will be highlighted by a press conference at the American Society for Cell Biology annual meeting to be held from December 13-18, 2013

(2) Peer-Reviewed Scientific Journals:
We will submit two manuscripts for publication this month. The first is entitled “Fidgetin-like 2 as a novel therapeutic target for wound healing” which will be submitted to Science Translational Medicine. The second is entitled “Non-mitotic functions of the centrosome scaffolding protein, Cep192” which will be submitted to PLOS*One*

(3) Invited Articles: None

(4) Abstracts: We will be presenting the following three abstracts at the American Society for Cell Biology Meeting
1) Fidgetin restrains axonal growth during neuronal maturation by a microtubule-based mechanism and provides a means for therapeutically enhancing regeneration of injured adult axons

2) Fidgetin-like 2 as a novel therapeutic target for wound healing
3) Non-mitotic functions of the centrosome scaffolding protein, Cep192

b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.
During the last year, I have made the following presentations of the work supported by this award:
1) Microtubule Mechanisms of Cell Migration, University of Pennsylvania, Muscle Institute, 2/13/13
2) Microtubules in Cell Migration and Wound healing, Gordon Research Conference: Motile and Contractile Systems, 8/1/2013
3) Microtubules in Cell Migration and Wound Healing, McGill University, Department of Pharmacology and Therapeutics, 10/28/13

6. **INVENTIONS, PATENTS AND LICENSES:**
This award has contributed to the filing of one patent application entitled: "Methods and Compositions to Inhibit Metastasis and to Treat Fibrosis and to Enhance Wound Healing", U.S. Provisional Application # 61/885,676
See appendix 1.

7. **REPORTABLE OUTCOMES:** Reportable outcomes are the same as "key research findings" above.

8. **OTHER ACHIEVEMENTS:** I have co-founded a company, MicroCures, which is built to commercialize the technology developed by this award. This award has also led to the generation of numerous antibodies which will be made available to the broader scientific community upon publication.

9. **REFERENCES:** None

10. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

NOTE:

TRAINING OR FELLOWSHIP AWARDS: For training or fellowship awards, in addition to the elements outlined above, include a brief description of opportunities for training and professional development. Training activities may include, for example, courses or one-on-one work with a mentor. Professional development activities may include workshops, conferences, seminars, and study groups.

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI

and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the GOR to obtain approval. REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE. It is the responsibility of the Principal Investigator to advise the GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. See term entitled "Intangible Property – Data and Software Requirements" and https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting for additional information.

Electronic Acknowledgement Receipt

EFS ID:	17020134
Application Number:	61885676
International Application Number:	
Confirmation Number:	3754
Title of Invention:	METHODS AND COMPOSITIONS TO INHIBIT METASTASIS AND TO TREAT FIBROSIS AND TO ENHANCE WOULD HEALING
First Named Inventor/Applicant Name:	David Sharp
Customer Number:	1912
Filer:	Brian Amos/Rosa Florio
Filer Authorized By:	Brian Amos
Attorney Docket Number:	96700/2051
Receipt Date:	02-OCT-2013
Filing Date:	
Time Stamp:	15:02:04
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$130
RAM confirmation Number	1477
Deposit Account	011785
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Provisional Cover Sheet (SB16)	1PTOSB16.pdf	32587 7765b5243a007e7bc4598b2f5289ced8fbf1ea4f	no	3
Warnings:					
This is not a USPTO supplied Provisional Cover Sheet SB16 form.					
Information:					
2	Specification	2spec.pdf	275850 36d7deb691bb6d8b40848f8fe9d472a94e6193d1	no	43
Warnings:					
Information:					
3	Claims	3claims.pdf	51355 1b251eb467e16ace319483c3b55b60d116ac9563	no	5
Warnings:					
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4	Abstract	4abstract.pdf	39519 dfb00b4d34cdff3a01ea072b519eeeeb68919469	no	1
Warnings:					
Information:					
5	Drawings-only black and white line drawings	5drawings.pdf	1693654 f2ed636082c123bc6545f5211ebce199106acddb	no	14
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	29720 79d574ce38a724a9c520e84eda45e40fb01330f4	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	2122685	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 01/31/2014 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1 — Remove

Given Name	Middle Name	Family Name	City	State	Country
David		Sharp	Scarsdale	NY	US

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Title of Invention	METHODS AND COMPOSITIONS TO INHIBIT METASTASIS AND TO TREAT FIBROSIS AND TO ENHANCE WOULD HEALING
Attorney Docket Number (if applicable)	96700/2051

Correspondence Address

Direct all correspondence to (select one):

◉ The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	01912

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

○ No.

◉ Yes, the name of the U.S. Government agency and the Government contract number are:

TATRC, USAMRMC W81XWH1210379

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Entity Status

Applicant claims small entity status under 37 CFR 1.27

- (●) Yes, applicant qualifies for small entity status under 37 CFR 1.27
- ( ) No

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Dkt. No. 96700/2051

METHODS AND COMPOSITIONS TO INHIBIT METASTASIS AND TO TREAT FIBROSIS AND TO ENHANCE WOUND HEALING

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made with government support under grant number W81XWH1210379 awarded by the Telemedicine and Advanced Technology Research Center (TATRC) at the U.S. Army Medical Research and Materiel Command (USAMRMC). The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0002] The disclosures of all publications, patents, patent application publications and books referred to in this application are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0003] Cancer metastasis is stimulated by the movement of cancer cells from the primary tumor to other tissues or organs. Metastatic cancer is responsible for the majority of cancer deaths. There are currently no effective means of treating metastasis, so the development of agents that inhibit the ability of cancer cells to move along their substrata for treating or inhibiting metastasis would represent a major advance.

[0004] Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. This results from the hyperproliferation and motility of cells, such as fibroblasts, that lay down connective tissue. Fibrosis can be a reactive, benign, or pathological state. In response to injury this is called scarring and if fibrosis arises from a single cell line this is called a fibroma. Physiologically this acts to deposit connective tissue, which can obliterate the architecture and function of the underlying organ or tissue. Fibrosis can be used to describe the pathological state of excess deposition of fibrous tissue, as well as the process of connective tissue deposition in healing. Fibrosis is similar to metastasis in that there are currently few therapeutic treatment strategies. The development of agents that prevent cell motility into wounded tissue would represent an important advance. Related to this, the development of safe and effective therapies for treating acute and chronic wounds is also of great interest. Wound healing is an intricate, multi-stage process that relies heavily on the delivery of new cells to the wound zone. Two key elements of the wound healing

response are fibroplasia and epithelialization when fibroblasts and epithelial cells, respectively, enter the wound to form a protective barrier from the external environment. This is stimulated by cell proliferation and migration from the wound edge. The identification of agents that increase the rate at which cells invade and close a wound would represent a major advance in wound healing therapeutics. Ideally, this would be a topically applied agent that stimulates the proliferation and migration of fibroblasts and wound edge epithelial cells.

[0005] The present invention addresses this need and identifies novel targets in treating and preventing metastasis, treating and preventing fibrosis, and treating and preventing pain associated with wound healing.

SUMMARY OF THE INVENTION

[0006] A method of treating metastasis or inhibiting metastasis in a subject having a cancer is provided comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat metastasis or inhibit metastasis.

[0007] Also provided is a method of treating metastasis or inhibiting metastasis in a subject having a cancer comprising administering to the subject an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat metastasis or inhibit metastasis.

[0008] Also provided is a method of treating fibrosis or scarring, or of inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat fibrosis or scarring, or inhibit fibrosis or scarring.

[0009] Also provided is a method of treating fibrosis or scarring, or inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat fibrosis or scarring, or inhibit fibrosis or scarring.

[0010] Also provided is a method of treating pain associated with wound healing in a subject having a wound comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat pain associated with wound healing.

[0011] Also provided is an inhibitor of KIF19, or of Kif19 gene product is provided for treating metastasis or inhibiting metastasis in a subject having a cancer.

[0012] Also provided is an inhibitor of CEP192 or of Cep192 gene product is provided for treating metastasis or inhibiting metastasis in a subject having a cancer.

[0013] Also provided is an inhibitor of KIF19, or of Kif19 gene product, is provided for treating fibrosis or scarring in a subject in need thereof.

[0014] Also provided is an inhibitor of CEP192 or of Cep192 gene product, is provided for treating fibrosis or scarring in a subject in need thereof.

[0015] Also provided is an inhibitor of CEP192 or of Cep192 gene product, for treating pain associated with wound healing in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1: A confocal micrograph showing a human U2OS cell double-labeled for Kif19 and the FA protein, vinculin. The far right panel is a higher magnification of the region boxed in "merge".

[0017] Fig. 2: The images show regions of U2OS cells (human Osteosarcoma) immunostained for the focal adhesion protein vinculin. The depletion of Kif19 by siRNA induces a substantial increase in the size and number of focal adhesions particularly in the cell interior.

[0018] Fig. 3A-3C: Panel A shows fluorescence recovery after photobleaching (FRAP) of GFP-vinculin labeled focal adhesions from control and Kif19 siRNA-treated U2OS cells. Time is shown in minutes: seconds. Panel B shows a representative fluorescence recovery plot from each condition. Panel C plots the density of focal adhesions in untreated cells (pre) and at various time points after nocodazole washout. Repolymerization of MTs after nocodazole washout was previously shown to stimulate focal adhesion disassembly (Ezratty, Partridge et al. 2005). The depletion of Kif19 prevents the disassembly of focal adhesions after nocodazole washout.

[0019] Fig. 4: Shows siRNA depletion of Kif19 decreases the motility of cancer cells *in vitro.*

[0020] Fig. 5: Shows an anaplastic thyroid carcinoma mouse model (Arciuch et al., Oncotarget, Dec. 2011). Dissociated tumor is removed from mouse and bathed in nanoparticles containing control or Kif19 siRNA for 2-24 hrs. Tumors are then embedded in matrigel and imaged daily. Movement of cells from tumor into matrigel is considered invasion. Black dots moving away from the dark central mass are invasive tumor cells. Kif19 nanoparticle siRNA treatment reduces tumor cell invasion relative to controls.

[0021] Fig. 6: Images showing the closure of control and Kif-19 siRNA-treated full thickness biopsy wounds positioned next to one another on the flank of a mouse. Images were taken 1 and 5 days after wounding. Kif19 siRNA or scrambled RNA (control) were encapsulated in nanoparticles and topically applied to wound.

[0022] Fig. 7: Time-series of TIRF images showing a field of fluorescently-labeled taxol-stabilized microtubules incubated with purified recombinant full-length Kif19. The time from the first to last image is 5 minutes.

[0023] Fig. 8A-8F: Kif19 is a microtubule depolymerase that localizes to substrate adhesions and promotes cell motility. A) Time series images of a fluorescent microtubule incubated with purified recombinant Kif19. B) Immunofluorescence showing the co-localization of Kif19 with the focal adhesion protein, vinculin. C) High magnification image showing a region of a cell double-labeled for Kif19 and microtubules. Microtubules often terminate at kif19-labeled substrate adhesions and these interactions are believed to control adhesion turnover rate. D) Control and Kif19 siRNA treated human U2OS cells labeled for the focal adhesion marker, vinculin. In cells depleted of Kif19, adhesions become significantly enlarged and hyperstable. E) Measured rates of *in vitro* wound closure in control and Kif19 siRNA-treated cultures (scratch assay). F) Movement trajectories of control and Kif19 siRNA-treated cells plotted from a common origin. The loss of Kif19 nearly completely suppresses cell movement.

[0024] Fig. 9: Confocal micrographs showing control and Cep192 siRNA-treated U20S cells immunolabeled for microtubules (red) and Cep192 (green). Cep192 siRNA treatment eliminates Cep192 immunofluorescence indicating a strong protein knockdown. Controls showed robust centrosomes with radial MT arrays while Cep192-depleted cells contained non-radial MT arrangements. Inset shows higher magnification of boxed region.

[0025] Fig. 10A-B: U2OS cells were treated with siRNA for 72 hours then exposed to 5uM nocodazole for 1 hour to depolymerize microtubules. Cells were then washed 3X with warm DMEM and then incubated for 10 minutes to allow microtubule regrowth. Images show control and Cep192 siRNA treated cells stained for microtubules. B) Control cells showed a significantly higher amount of regrowth from the centrosome than did cells depleted of Cep192; $P < 0.0001$. S.E.M. is depicted as vertical bars.

[0026] Fig. 11A-10D: Figure 10A-10D: A) Time-lapse phase-contrast images of control and Cep192 siRNA treated U2OS cells from an *in vitro* wound healing assay. U2OS cells

were plated into Ibidi Culture-Insert dishes following knockdown. B) Significantly fewer Cep192 depleted cells entered the wound zone relative to controls. P<0.0001. S.E.M. is depicted as vertical bars. C) Time-lapse phase-contrast images of control and Cep192 siRNA-treated HEKa (human epidermal keratinoctyes - adult) cells from an *in vitro* wound healing assay. HEKa cells were plated into Ibidi Culture-Insert dishes following. D) Significantly fewer Cep192-depleted cells entered the wound zone relative to controls. P<0.0001. S.E.M. is depicted as vertical bars.

[0027] Fig. 12: Anaplastic thyroid carcinoma invasion assay: a dissociated tumor is removed from mouse and bathed in nanoparticles for 2 hrs. (48 hours) (top Panels). The tumor is embedded in Matrigel/Collagen matrix and imaged daily (96 hours) (bottom panels).

[0028] Fig. 13: Human large cell lung tumors. SC injection of human H460 lung cancer cells into mice (top panels). Invasive tumors (1-2 cm) removed and bathed in nanoparticles for 2 hrs. (middle panels). Tumor embedded in Matrigel/Collagen matrix and imaged daily (bottom panels).

[0029] Fig. 14: Fidgetin and Cep192 regulate axon regeneration. Images are immunofluorescence micrographs of primary adult rat DRG neurons treated with control, Fidgetin or Cep192 nanoparticle encapsulated siRNA. Cells were fixed 24 hours after plating and siRNA treatment. Bottom right panel shows the average axon length in each condition (longest process from each individual cell was measured; error bars are SEM). ***P<0.01; **P<0.05.

DETAILED DESCRIPTION OF THE INVENTION

[0030] A method of treating metastasis or inhibiting metastasis in a subject having a cancer is provided comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat metastasis or inhibit metastasis.

[0031] As used herein, “treating” metastasis means ameliorating or lessening or reducing further progression of an extant metastasis. As used herein, “inhibiting” metastasis means lessening the extent of, development of, or progression of a metastasis.

[0032] In embodiments of the invention described herein, the preferred subject is a human subject.

[0033] Also provided is a method of treating metastasis or inhibiting metastasis in a subject having a cancer comprising administering to the subject an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat metastasis or inhibit metastasis.

[0034] Also provided is a method of treating fibrosis or scarring, or of inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat fibrosis or scarring, or inhibit fibrosis or scarring. As used herein, "treating" a fibrosis means ameliorating or lessening or reducing further progression of an extant fibrosis. As used herein, "inhibiting" fibrosis means lessening the extent of, development of, or progression of a fibrosis. As used herein, "treating" scarring means ameliorating or lessening or reducing further progression of an extant scarring or scarring process. As used herein, "inhibiting" scarring means lessening the extent of, development of, or progression of a scarring or scarring process.

[0035] As used herein, any recitation of embodiments in the alternative, e.g. embodiment A or embodiment B, includes the specific, separate embodiments of (i) embodiment A and (ii) of embodiment B, as part of the invention.

[0036] Also provided is a method of treating fibrosis or scarring, or inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat fibrosis or scarring, or inhibit fibrosis or scarring.

[0037] Also provided is a method of treating pain associated with wound healing in a subject having a wound comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat pain associated with wound healing. As used herein, "treating" pain associated with wound healing means ameliorating or lessening or reducing pain associated with an extant wound.

[0038] In an embodiment of the methods, the KIF19 or Kif19 gene product is a human KIF19 or human Kif19 gene product, respectively.

[0039] In an embodiment of the methods, the CEP192 or Cep192 gene product is a human CEP192 or a human Cep192 gene product, respectively.

[0040] In an embodiment of the methods, the inhibitor of KIF19 is an RNAi nucleic acid. In an embodiment of the methods, the inhibitor of CEP192 is an RNAi nucleic acid. In an embodiment of the methods, the RNAi nucleic acid is a siRNA directed to KIF19 or a shRNA directed to KIF19. In an embodiment of the methods, the RNAi nucleic acid is a

siRNA directed to CEP192 or a shRNA directed to CEP192. In an embodiment of the methods, the siRNA is administered. In an embodiment of the methods, the shRNA is administered. In an embodiment of the methods, the siRNA is administered as a composition comprising the siRNA associated with a nanoparticle. In an embodiment of the methods, the siRNA is administered as a composition comprising the siRNA encapsulated with a nanoparticle. In an embodiment of the methods, the nanoparticle is PEGylated. In an embodiment of the methods, the siRNA is administered as a viral vector. In an embodiment of the methods, the shRNA is administered as a viral vector.

[0041] In an embodiment of the methods, the cancer is a thyroid, blood, bladder, breast, colorectal, kidney, lung, melanoma, ovary, pancreas, prostate or stomach cancer. In an embodiment of the methods, the cancer is an anaplastic thyroid carcinoma. In an embodiment of the methods, the cancer is large cell lung cancer.

[0042] In an embodiment of the methods, the fibrosis is in response to an injury. In an embodiment of the methods, the fibrosis is a fibroma, pulmonary fibrosis, cystic fibrosis, hepatic cirrhosis, endomyocardial fibrosis, from a previous myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis of the lungs, a complication of pneumoconiosis, nephrogenic systemic fibrosis, Crohn's disease fibrosis, keloid fibrosis, scleroderma/systemic sclerosis of skin or lungs, arthrofibrosis or adhesive capsulitis fibrosis.

[0043] In an embodiment of the methods, the scarring is skin scarring, cardiovascular scarring, or neuronal scarring.

[0044] In an embodiment of the methods, the wound is a skin wound, cardiovascular wound, or neuronal wound. In an embodiment of the methods, the skin wound is a burn wound.

[0045] In an embodiment of the methods regarding wounds, scarring or treating pain associated with the wound, the inhibitor may be applied to the skin of the subject.

[0046] Also provided is a method of identifying an anti-metastatic agent comprising contacting a nucleic acid encoding Kif19 gene product with the agent or contacting Kif19 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Kif19 gene product or inhibits activity of the Kif19 gene product, respectively, and subsequently identifying the agent as an anti-metastatic agent or not,

wherein an agent that inhibits Kif19 expression or Kif19 gene product is identified as an anti-metastatic agent.

[0047] Preferably, an "agent" in the methods of identifying an anti-metastatic agent, anti-fibrotic agent, or pain-relieving agent, is a small organic molecule of 1,500 daltons or less, a peptide, a protein, an antibody, a fragment of an antibody, a carbohydrate, an oligonucleotide or a nucleic acid. In an embodiment of the methods of identifying an agent as set forth herein, the agent is a small organic molecule, a peptide, a nucleic acid, an oligonucleotide, an antibody, an antigen-binding fragment of an antibody or an aptamer.

[0048] Also provided is a method of identifying an anti-metastatic agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and subsequently identifying the agent as an anti-metastatic agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as an anti-metastatic agent.

[0049] Also provided is a method of identifying an anti-fibrotic agent comprising contacting a nucleic acid encoding Kif19 gene product with the agent or contacting Kif19 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Kif19 gene product or inhibits activity of the Kif19 gene product, respectively, and subsequently identifying the agent as an anti-fibrotic agent or not, wherein an agent that inhibits Kif19 expression or Kif19 gene product is identified as an anti-fibrotic agent.

[0050] Also provided is a method of identifying an anti-fibrotic agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and subsequently identifying the agent as an anti-fibrotic agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as an anti-fibrotic agent.

[0051] Also provided is a method of identifying a pain-relieving agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the

nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and subsequently identifying the agent as a pain-relieving agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as a pain-relieving agent.

[0052] Generally herein, with regard to KIF19 and Kif19, "KIF19" (i.e. upper case) refers to the gene and "Kif19" (i.e. lower case) refers to the protein. The protein may also be referred to as "Kif19 gene product." Generally herein, with regard to CEP192 and Cep192, "CEP192" (i.e. upper case) refers to the gene and "Cep192" (i.e. lower case) refers to the protein. The protein may also be referred to as "Cep192 gene product." As used herein, a transcript of a given gene means any nucleic acid, for example an mRNA, that encodes the protein gene product encoded by the gene. Thus, a transcript of CEP192 includes an mRNA encoding CEP192 gene product. Thus, a transcript of KIF19 includes an mRNA encoding KIF19 gene product.

[0053] A pharmaceutical composition is provided comprising an amount of an inhibitor of KIF19 or of Kif19 gene product. In an embodiment, the pharmaceutical composition comprises an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat a wound in a human subject, or comprises an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat or inhibit metastasis in a subject, or comprises an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat or inhibit fibrosis in a subject. In an embodiment, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In an embodiment of the pharmaceutical composition, the inhibitor of KIF19 or of Kif19 gene product is encapsulated, completely or partially, by a nanoparticle. In an embodiment the nanoparticle comprises a hydrogel/sugar glass composite. In an embodiment, the nanoparticle is PEGylated. In an embodiment the nanoparticle is a liposomal nanoparticle. In an embodiment, the nanoparticle is paramagnetic. In an embodiment of the methods and compositions, the inhibitor is an siRNA which inhibits expression of Kif19 gene product. In an embodiment, the inhibitor is an shRNA which inhibits expression of Kif19 gene product.

[0054] The optimal dosage of the KIF19 inhibitor or of Kif19 gene product inhibitor administered in treatments herein will vary depending upon factors such as the pharmacodynamic characteristics of a specific inhibitor and its mode and route of administration; the age, sex, metabolic rate, absorptive efficiency, health and weight of the

recipient; the nature and extent of the symptoms; the kind of concurrent treatment being administered; the frequency of treatment with the inhibitor and the desired therapeutic effect. A dosage unit of the KIF19 inhibitor or of Kif19 gene product inhibitor may comprise a single compound, or a mixture of the compound with one or more anti-infection compound(s) or wound healing-promoting compound(s); one or more anti-cancer compounds; or one or more anti-fibrotic compounds, as relevant to the condition being treated.

[0055] In an embodiment of the methods or compositions, inhibition is effected by RNAi. In an embodiment, RNAi inhibition of KIF19 or of Kif19 gene product expression is effected with an siRNA. The siRNA (small interfering RNA) as used in the methods or compositions described herein comprises a portion which is complementary to a nucleic acid, in a non-limiting example an mRNA, sequence encoding a Kif19 gene product. In an embodiment, the Kif19 gene product is a human Kif19 gene product. In an embodiment, the mRNA is or is encoded by NCBI Reference Sequence: NM_153209.3 (SEQ ID NO:1), and the siRNA is effective to inhibit expression of Kif19 gene product. In an embodiment, the mRNA is or is encoded by a known variant of the NCBI Reference Sequence: NM_153209.3 (SEQ ID NO:1), and the siRNA is effective to inhibit expression of Kif19 gene product. In an embodiment, the Kif19 gene product comprises consecutive amino acid residues having the sequence set forth in SEQ ID NO:2.

[0056] In an embodiment, the siRNA comprises a double-stranded portion (duplex). In an embodiment, the siRNA is 20-25 nucleotides in length. In an embodiment the siRNA comprises a 19-21 core RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. The siRNA can be 5' phosphorylated, or not, and may be modified with any of the known modifications in the art to improve efficacy and/or resistance to nuclease degradation. In an embodiment, the siRNA is 5' phosphorylated. In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In one embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the double-stranded RNA is 80%, 85%, 90%, 95% or 100% complementary to a portion of an RNA transcript of a KIF19 (gene) encoding Kif19 gene product. In an embodiment, the RNA transcript of a gene encoding Kif19 gene product is an mRNA. In an embodiment, the Kif19 gene product is a human Kif19 gene product.

[0057] In an embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the RNA comprises a portion having a sequence the same as a portion of 18-25 consecutive nucleotides of an RNA transcript of a gene encoding Kif19 gene product. In an embodiment, the other strand is fully complementary to the one strand. In an embodiment, the Kif19 gene product is a human Kif19 gene product. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a non-nucleotide linker. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein the two strands of RNA are not connected other than by complementary hybridization. Alternately, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a nucleotide linker, such as a loop or stem loop structure. In an embodiment, one strand of the double-stranded siRNA is fully complementary to a nucleic acid encoding Kif19 gene product. In an embodiment, one strand of the double-stranded siRNA is fully complementary to a nucleic acid encoding Kif19 gene product except at one, or except at two, mismatched positions. In one embodiment, a single strand component of a siRNA of the invention is from 14 to 50 nucleotides in length. In another embodiment, a single strand component of a siRNA of the invention is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 18 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 19 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 20 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 21 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 22 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 23 nucleotides in length. In one embodiment, a siRNA of the invention is from 28 to 56 nucleotides in length. In another embodiment, a siRNA of the invention is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 nucleotides in length. In another embodiment, an siRNA of the invention comprises at least one 2'-sugar modification. In an embodiment, an siRNA of the invention comprises at least one nucleic acid base modification. In an embodiment, an siRNA of the invention comprises at least one phosphate backbone modification. As used herein, “at least one” means one or more. In an embodiment, the double-stranded siRNA of the invention comprises an

overhang of one or two nucleotides. In an embodiment, the overhang is a 3' overhang. In an embodiment, the overhang is a 5' overhang. In an embodiment, the overhang is a 3' overhang of two nucleotides. In an embodiment, the overhang is one of UU, UG or dTdT. In an embodiment, the double-stranded siRNA of the invention comprises an overhang of one or two nucleotides on each of its two strands. In an embodiment, the two overhangs are 3' overhangs. In an embodiment, the two overhangs are of one nucleotide each. In an embodiment, the two overhangs are of two nucleotides each. In an embodiment, the overhangs are one of UU, UG or dTdT. In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated.

[0058] In one embodiment, RNAi inhibition of KIF19 or of Kif19 gene product expression is effected by a short hairpin RNA ("shRNA"). The shRNA is introduced into the appropriate cell by transduction with a vector. In an embodiment, the vector is a lentiviral vector. In an embodiment, the vector comprises a promoter. In an embodiment, the promoter is a U6 or H1 promoter. In an embodiment the shRNA encoded by the vector is a first nucleotide sequence ranging from 19-29 nucleotides complementary to the target gene/mRNA, in the present case the mRNA encodes Kif19 gene product. In an embodiment the Kif19 gene product is a human Kif19 gene product. In an embodiment the shRNA encoded by the vector also comprises a short spacer of 4-15 nucleotides (a loop, which does not hybridize) and a 19-29 nucleotide sequence that is a reverse complement of the first nucleotide sequence. In an embodiment the siRNA resulting from intracellular processing of the shRNA has overhangs of 1 or 2 nucleotides. In an embodiment the siRNA resulting from intracellular processing of the shRNA overhangs has two 3' overhangs. In an embodiment the overhangs are, independently, UU, UG or dTdT.

[0059] In a preferred embodiment, the inhibitor of KIF19 or of Kif19 gene product expression is an siRNA. In a preferred embodiment the siRNA is encapsulated in a nanoparticle. In an embodiment, the nanoparticle comprises a hydrogel/sugar glass composite. In an embodiment the nanoparticle is a liposomal nanoparticle. In an embodiment, the nanoparticle is PEGylated. In embodiments the PEG is PEG-500 or PEG-3000 or PEG-5000. In an embodiment, the nanoparticle is doped with amino silanes. In an embodiment, the nanoparticle is paramagnetic.

[0060] As used herein an “aptamer”, with regard to KIF19 or Kif19, is a single-stranded oligonucleotide or oligonucleotide analog that binds to a Kif19 gene product, or to a nucleic acid (such as KIF19) encoding a Kif19 gene product, and inhibits the function or expression thereof, as appropriate.

[0061] The present invention provides kits for treating wounds or scarring, a kit for treating or inhibiting metastasis, a kit for treating or inhibiting fibrosis, the kit comprising an inhibitor of KIF19 or an inhibitor of Kif19.

[0062] A composition provided in such a kit for treating or inhibiting metastasis may be provided in a form suitable for reconstitution prior to use (such as a lyophilized injectable composition) or in a form which is suitable for immediate application by, for example, injection, such as an aqueous composition.

[0063] A composition provided in such a kit for treating wounds or scarring may be provided in a form suitable for reconstitution prior to use (such as a lyophilized injectable composition) or in a form which is suitable for immediate application to a wound, including to the wound margin, such as a lotion or ointment. In an embodiment for treating wounds, the inhibitor of KIF19 or of Kif19 gene product is administered locally to the wound.

[0064] In an embodiment, the inhibitor of KIF19 or of Kif19 product is administered via a vein or artery. In an embodiment, the inhibitor of KIF19 or of Kif19 gene product is administered by injection, catheterization or cannulation.

[0065] In an embodiment, the inhibitor of KIF19 or of Kif19 gene product is administered from an implant that elutes the inhibitor, for example a eluting stent or an eluting skin patch.

[0066] In an embodiment, the wound is an epidermal wound. In an embodiment, the wound is a skin wound. In an embodiment, the wound is a cardiac tissue wound. In an embodiment, the wound is a cardiovascular wound, for example resulting from a myocardial infarction. In an embodiment, the wound is a neuronal wound. In an embodiment for treating wounds, the inhibitor of Kif19 is provided by a subcutaneous implant or depot medicament system for the pulsatile delivery of the inhibitor to a wound or site where a wound is to expected be formed to promote wound healing. The inhibitor can be provided, for example, in a therapeutically effective amount to each centimeter of a wound margin or each centimeter of a site at which a wound is expected to be formed. The benefits that may be derived from the present invention may be applicable to wounds at

sites throughout the body. However, it may be preferred that the wound for which healing is promoted is a skin wound. For illustrative purposes the embodiments of the invention will generally be described with reference to skin wounds, although they remain applicable to other tissues and organs. Merely by way of example, in another preferred embodiment the wound may be a wound of the circulatory system, particularly of a blood vessel. Other wounds in which wound healing may be promoted in accordance with the present invention include as a result of surgery or as a result of a burn. Other wounds in which wound healing may be promoted in accordance with the present invention include skin ulcers caused by pressure, venous stasis, or diabetes mellitus. Examples of specific wounds in which healing may be promoted using the medicaments and methods of treating wounds or promoting healing of wounds described herein include, but are not limited to, those independently selected from the group consisting of: wounds of the skin; wounds of the eye (including the inhibition of scarring resulting from eye surgery such as LASIK surgery, LASEK surgery, PRK surgery, glaucoma filtration surgery, cataract surgery, or surgery in which the lens capsule may be subject to scarring) such as those giving rise to corneal cicatrisation; wounds subject to capsular contraction (which is common surrounding breast implants); wounds of blood vessels; wounds of the central and peripheral nervous system (where prevention, reduction or inhibition of scarring may enhance neuronal reconnection and/or neuronal function); wounds of tendons, ligaments or muscle; wounds of the oral cavity, including the lips and palate (for example, to inhibit scarring resulting from treatment of cleft lip or palate); wounds of the internal organs such as the liver, heart, brain, digestive tissues and reproductive tissues; wounds of body cavities such as the abdominal cavity, pelvic cavity and thoracic cavity (where inhibition of scarring may reduce the number of incidences of adhesion formation and/or the size of adhesions formed); and surgical wounds (in particular wounds associated with cosmetic procedures, such as scar revision). It is particularly preferred that the medicaments and methods of the invention regarding wounds be used to promote healing of wounds of the skin.

[0067] A medicament in accordance with this aspect of the invention may be formulated in any appropriate carrier. Suitable carriers are pharmaceutically acceptable carriers, for example, preferably those consistent with administration topically or administration by injection for treating wounds and treating or preventing fibrosis; preferably those consistent with administration intravenously or administration by injection or cannulation for treating

or preventing metastasis. It will be appreciated that, while the inhibitor of Kif19 may be administered by the same route and in the same form in each incidence of treatment, different incidences of treatment may provide the inhibitor of Kif19 by different medicaments and/or different routes of administration. In embodiments of the invention the initial incidence of treatment may provide the inhibitor of Kif19 by means of an injection, such as an intradermal injection, while the second (and any subsequent) incidences of treatment may involve provision of the inhibitor of Kif19 by alternative routes, such as topical formulations, or vice versa. In an embodiment, multiple administrations of the inhibitor of Kif19 may be effected by the same means or route. In an embodiment the shRNA or siRNA inhibitor of Kif19 can be administered such that it is transfected into one or more cells.

[0068] In a non-limiting embodiment the inhibitor of KIF 19 or Kif19 is provided in a bulk-eroding system such as polylactic acid and glycolic acid (PLGA) copolymer based microspheres or microcapsules systems containing the inhibitor of Kif19. In an embodiment, blends of PLGA:ethylcellulose systems may be used as an appropriate carrier. A further medicament in accordance with this aspect of the invention may be formulated in a surface-eroding system wherein the inhibitor of Kif19 or of KIF19 is embedded in an erodible matrix such as the poly(ortho) ester and polyanhydride matrices wherein the hydrolysis of the polymer is rapid. A medicament in accordance with this aspect of the invention may also be formulated by combining a pulsatile delivery system as described above and an immediate release system such as a lyophilized injectable composition described above.

[0069] The inhibitor may be used in a composition with additives. Examples of suitable additives are sodium alginate, as a gelatinizing agent for preparing a suitable base, or cellulose derivatives, such as guar or xanthan gum, inorganic gelatinizing agents, such as aluminum hydroxide or bentonites (termed thixotropic gel-formers), polyacrylic acid derivatives, such as Carbopol®, polyvinylpyrrolidone, microcrystalline cellulose and carboxymethylcellulose. Amphiphilic low molecular weight and higher molecular weight compounds, and also phospholipids, are also suitable. The gels can be present either as water-based hydrogels or as hydrophobic organogels, for example based on mixtures of low and high molecular weight paraffin hydrocarbons and vaseline. The hydrophilic organogels can be prepared, for example, on the basis of high molecular weight polyethylene glycols.

These gelatinous forms are washable. Hydrophobic organogels are also suitable. Hydrophobic additives, such as petroleum jelly, wax, oleyl alcohol, propylene glycol monostearate and/or propylene glycol monopalmitostearate, in particular isopropyl myristate can be included. In an embodiment the inhibitor is in a composition comprising one or more dyes, for example yellow and/or red iron oxide and/or titanium dioxide for the purpose of matching as regards color. Compositions may be in any suitable form including gels, lotions, balms, pastes, sprays, powders, bandages, wound dressing, emulsions, creams and ointments of the mixed-phase or amphiphilic emulsion systems (oil/water-water/oil mixed phase), liposomes and transfersomes or plasters/band aid-type coverings. Emulsifiers which can be employed in compositions comprising the inhibitor of KIF19 or of Kif19 include anionic, cationic or neutral surfactants, for example alkali metal soaps, metal soaps, amine soaps, sulphurated and sulphonated compounds, invert soaps, higher fatty alcohols, partial fatty acid esters of sorbitan and polyoxyethylene sorbitan, e.g. lanette types, wool wax, lanolin or other synthetic products for preparing the oil/water and/or water/oil emulsions.

[0070] Compositions comprising the inhibitor of Kif19 can also comprise vaseline, natural or synthetic waxes, fatty acids, fatty alcohols, fatty acid esters, for example as monoglycerides, diglycerides or triglycerides, paraffin oil or vegetable oils, hydrogenated castor oil or coconut oil, hog fat, synthetic fats (for example based on caprylic acid, capric acid, lauric acid or stearic acid, such as Softisan®), or triglyceride mixtures, such as Miglyol®, can be used as lipids, in the form of fatty and/or oleaginous and/or waxy components for preparing the ointments, creams or emulsions of the compositions comprising the inhibitor of Kif19 used in the methods described herein.

[0071] Osmotically active acids and alkaline solutions, for example hydrochloric acid, citric acid, sodium hydroxide solution, potassium hydroxide solution, sodium hydrogen carbonate, may also be ingredients of the compositions of the invention and, in addition, buffer systems, such as citrate, phosphate, tris buffer or triethanolamine, for adjusting the pH. It is possible to add preservatives as well, such as methyl benzoate or propyl benzoate (parabens) or sorbic acid, for increasing the stability.

[0072] Pastes, powders and solutions are additional forms of compositions comprising the inhibitor of Kif19 which can be applied topically. As consistency-imparting bases, the pastes frequently contain hydrophobic and hydrophilic auxiliary substances, preferably,

however, hydrophobic auxiliary substances containing a very high proportion of solids. In order to increase dispersity, and also flowability and slipperiness, and also to prevent agglomerates, the powders or topically applicable powders can, for example, contain starch species, such as wheat or rice starch, flame-dispersed silicon dioxide or siliceous earth, which also serve as diluent.

[0073] A method is provided for identifying a candidate agent for treating a wound comprising:

a) determining the activity of an amount of Kif19 gene product; and

b) contacting the amount of Kif19 gene product with the candidate agent and determining the activity of the amount of Kif19 gene product in the presence of the candidate agent,

wherein a decreased activity of the amount of Kif19 gene product in the presence of the candidate agent as compared to the activity of Kif19 gene product in the absence of the candidate agent indicates that the candidate agent can treat a wound, and wherein no change in or an increased activity of the amount of Kif19 gene product in the presence of the candidate agent as compared to the activity of Kif19 gene product in the absence of the candidate agent does not indicate that the candidate agent can treat a wound. In an embodiment, the candidate agent is a small molecule of 2000 Daltons or less. In an embodiment, the candidate agent is a small molecule of 1000 Daltons or less. In an embodiment, the candidate agent is a small molecule of 1500 Daltons or less. In an embodiment, the candidate agent is a substituted or un-substituted hydrocarbon small molecule. In an embodiment, the inhibitor or the candidate agent is an aptamer, a nucleic acid, an oligonucleotide, or a small organic molecule of 2000 Daltons or less. In an embodiment, the inhibitor is cell-membrane permeable.

[0074] A pharmaceutical composition is provided comprising an amount of an inhibitor of CEP192 or of Cep192 gene product. In an embodiment, the pharmaceutical composition comprises an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat a wound in a human subject, or comprises an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat or inhibit metastasis in a subject, or comprises an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat or inhibit fibrosis in a subject, or comprises an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat or inhibit pain associated with a wound or wound healing in a

subject. In an embodiment, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In an embodiment of the pharmaceutical composition, the inhibitor of CEP192 or of Cep192 gene product is encapsulated, completely or partially, by a nanoparticle. In an embodiment the nanoparticle comprises a hydrogel/sugar glass composite. In an embodiment, the nanoparticle is PEGylated. In an embodiment the nanoparticle is a liposomal nanoparticle. In an embodiment, the nanoparticle is paramagnetic. In an embodiment of the methods and compositions, the inhibitor is an siRNA which inhibits expression of Cep192 gene product. In an embodiment, the inhibitor is an shRNA which inhibits expression of Cep192 gene product.

[0075] The optimal dosage of the CEP192 inhibitor or of Cep192 gene product inhibitor administered in treatments herein will vary depending upon factors such as the pharmacodynamic characteristics of a specific inhibitor and its mode and route of administration; the age, sex, metabolic rate, absorptive efficiency, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment being administered; the frequency of treatment with the inhibitor and the desired therapeutic effect. A dosage unit of the CEP192 inhibitor or of Cep192 gene product inhibitor may comprise a single compound, or a mixture of the compound with one or more anti-infection compound(s) or wound healing-promoting compound(s); one or more anti-cancer compounds; or one or more anti-fibrotic compounds; or one or more pain-relieveing compounds, as relevant to the condition being treated.

[0076] In an embodiment of the methods or compositions, inhibition of CEP192 or of Cep192 is effected by RNAi. In an embodiment, RNAi inhibition of CEP192 or of Cep192 gene product expression is effected with an siRNA. The siRNA (small interfering RNA) as used in the methods or compositions described herein comprises a portion which is complementary to a nucleic acid, in a non-limiting example an mRNA, sequence encoding a Cep192 gene product. In an embodiment, the Cep192 gene product is a human Cep192 gene product. In an embodiment, the mRNA is or is encoded by NCBI Reference Sequence: NM_032142.3 (SEQ ID NO:3), and the siRNA is effective to inhibit expression of Cep192 gene product. In an embodiment, the mRNA is or is encoded by a known variant of NCBI Reference Sequence: NM_032142.3 (SEQ ID NO:3), and the siRNA is effective to inhibit expression of Cep192 gene product. In an embodiment, the Cep192 gene product comprises consecutive amino acid residues having the sequence set forth in SEQ ID NO:4.

[0077] In an embodiment, the siRNA comprises a double-stranded portion (duplex). In an embodiment, the siRNA is 20-25 nucleotides in length. In an embodiment the siRNA comprises a 19-21 core RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. The siRNA can be 5' phosphorylated, or not, and may be modified with any of the known modifications in the art to improve efficacy and/or resistance to nuclease degradation. In an embodiment, the siRNA is 5' phosphorylated. In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In one embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the double-stranded RNA is 80%, 85%, 90%, 95% or 100% complementary to a portion of an RNA transcript of a CEP192 (gene) encoding Cep192 gene product. In an embodiment, the RNA transcript of a gene encoding Cep192 gene product is an mRNA. In an embodiment, the Cep192 gene product is a human Cep192 gene product.

[0078] In an embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the RNA comprises a portion having a sequence the same as a portion of 18-25 consecutive nucleotides of an RNA transcript of a gene encoding Cep192 gene product. In an embodiment, the other strand is fully complementary to the one strand. In an embodiment, the Cep192 gene product is a human Cep192 gene product. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a non-nucleotide linker. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein the two strands of RNA are not connected other than by complementary hybridization. Alternately, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a nucleotide linker, such as a loop or stem loop structure. In an embodiment, one strand of the double-stranded siRNA is fully complementary to a nucleic acid encoding Cep192 gene product. In an embodiment, one strand of the double-stranded siRNA is fully complementary to a nucleic acid encoding Cep192 gene product except at one, or except at two, mismatched positions. In one embodiment, a single strand component of a siRNA of the invention is from 14 to 50 nucleotides in length. In another embodiment, a single strand component of a siRNA of the invention is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides in length. In an embodiment, a single strand component of a siRNA of the invention is 18 nucleotides in length. In yet another embodiment, a single strand

component of a siRNA of the invention is 19 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 20 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 21 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 22 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 23 nucleotides in length. In one embodiment, a siRNA of the invention is from 28 to 56 nucleotides in length. In another embodiment, a siRNA of the invention is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 nucleotides in length. In an embodiment, an siRNA of the invention comprises at least one 2'-sugar modification. In an embodiment, an siRNA of the invention comprises at least one nucleic acid base modification. In an embodiment, an siRNA of the invention comprises at least one phosphate backbone modification. As used herein, "at least one" means one or more. In an embodiment, the double-stranded siRNA of the invention comprises an overhang of one or two nucleotides. In an embodiment, the overhang is a 3' overhang. In an embodiment, the overhang is a 5' overhang. In an embodiment, the overhang is a 3' overhang of two nucleotides. In an embodiment, the overhang is one of UU, UG or dTdT. In an embodiment, the double-stranded siRNA of the invention comprises an overhang of one or two nucleotides on each of its two strands. In an embodiment, the two overhangs are 3' overhangs. In an embodiment, the two overhangs are of one nucleotide each. In an embodiment, the two overhangs are of two nucleotides each. In an embodiment, the overhangs are, independently, one of UU, UG or dTdT. In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated.

[0079] In one embodiment, RNAi inhibition of CEP192 or of Cep192 gene product expression is effected by a short hairpin RNA ("shRNA"). The shRNA is introduced into the appropriate cell by transduction with a vector. In an embodiment, the vector is a lentiviral vector. In an embodiment, the vector comprises a promoter. In an embodiment, the promoter is a U6 or H1 promoter. In an embodiment the shRNA encoded by the vector is a first nucleotide sequence ranging from 19-29 nucleotides complementary to the target gene/mRNA, in the present case the mRNA encodes Cep192 gene product. In an embodiment the Cep192 gene product is a human Cep192 gene product. In an embodiment the shRNA encoded by the vector also comprises a short spacer of 4-15 nucleotides (a loop,

which does not hybridize) and a 19-29 nucleotide sequence that is a reverse complement of the first nucleotide sequence. In an embodiment the siRNA resulting from intracellular processing of the shRNA has overhangs of 1 or 2 nucleotides. In an embodiment the siRNA resulting from intracellular processing of the shRNA overhangs has two 3' overhangs. In an embodiment the overhangs are, independently, UU, UG or dTdT.

[0080] As used herein an "aptamer", with regard to CEP192 or Cep192, is a single-stranded oligonucleotide or oligonucleotide analog that binds to a Cep192 gene product, or to a nucleic acid (such as CEP192) encoding a Cep192 gene product, and inhibits the function or expression thereof, as appropriate.

[0081] The present invention provides kits for treating wounds or scarring, a kit for treating or inhibiting metastasis, a kit for treating or inhibiting fibrosis, or a kit for treating or inhibiting pain associated with a wound or with wound healing, the kit comprising an inhibitor of CEP192 or an inhibitor of Cep192.

[0082] A composition provided in such a kit for treating or inhibiting metastasis may be provided in a form suitable for reconstitution prior to use (such as a lyophilized injectable composition) or in a form which is suitable for immediate application by, for example, injection, such as an aqueous composition.

[0083] A composition provided in such a kit for treating or inhibiting pain associated with a wound or with wound healing, may be provided in a form suitable for reconstitution prior to use (such as a lyophilized injectable composition) or in a form which is suitable for immediate application by, for example, injection, such as an aqueous composition, or a form for immediate topical application, such as a lotion or ointment.

[0084] A composition provided in such a kit for treating wounds or scarring may be provided in a form suitable for reconstitution prior to use (such as a lyophilized injectable composition) or in a form which is suitable for immediate application to a wound, including to the wound margin, such as a lotion or ointment. In an embodiment for treating wounds, the inhibitor of CEP192 or of Cep192 gene product is administered locally to the wound.

[0085] In an embodiment, the inhibitor of CEP192 or of Cep192 product is administered via a vein or artery. In an embodiment, the inhibitor of CEP192 or of Cep192 gene product is administered by injection, catheterization or cannulation.

[0086] In an embodiment, the inhibitor of CEP192 or of Cep192 gene product is administered from an implant that elutes the inhibitor, for example a eluting stent or an eluting skin patch.

[0087] In an embodiment, the wound is an epidermal wound. In an embodiment, the wound is a skin wound. In an embodiment, the wound is a cardiac tissue wound. In an embodiment, the wound is a cardiovascular wound, for example resulting from a myocardial infarction. In an embodiment, the wound is a neuronal wound. In an embodiment of the invention the inhibitor of Cep192 is provided by a subcutaneous implant or depot medicament system for the pulsatile delivery of the inhibitor to a wound or site where a wound is to expected be formed to promote wound healing. The inhibitor can be provided, for example, in a therapeutically effective amount to each centimeter of a wound margin or each centimeter of a site at which a wound is expected to be formed. The benefits that may be derived from the present invention may be applicable to wounds at sites throughout the body. However, it may be preferred that the wound for which healing is promoted is a skin wound. For illustrative purposes the embodiments of the invention will generally be described with reference to skin wounds, although they remain applicable to other tissues and organs. Merely by way of example, in another preferred embodiment the wound may be a wound of the circulatory system, particularly of a blood vessel. Other wounds in which wound healing may be promoted in accordance with the present invention include as a result of surgery or as a result of a burn. Other wounds in which wound healing may be promoted in accordance with the present invention include skin ulcers caused by pressure, venous stasis, or diabetes mellitus. In an embodiment, the inhibitor of CEP192 or of Cep192 gene product is administered locally to the wound. In an embodiment, the inhibitor of CEP192 or of Cep192 gene product is administered via a vein or artery. In an embodiment, the inhibitor of CEP19 or of Cep19 gene product is administered by injection, catheterization or cannulation. In an embodiment, the inhibitor of CEP192 or of Cep19 gene product is administered from an implant that elutes the inhibitor, for example a eluting stent or an eluting skin patch. In an embodiment, the wound is an epidermal wound. In an embodiment, the wound is a skin wound. In an embodiment, the wound is a cardiac tissue wound. In an embodiment, the wound is a cardiovascular wound, for example resulting from a myocardial infarction. In an embodiment, the wound is a neuronal wound. Examples of specific wounds in which healing may be promoted using the medicaments and methods

of treating wounds or promoting healing of wounds described herein include, but are not limited to, those independently selected from the group consisting of: wounds of the skin; wounds of the eye (including the inhibition of scarring resulting from eye surgery such as LASIK surgery, LASEK surgery, PRK surgery, glaucoma filtration surgery, cataract surgery, or surgery in which the lens capsule may be subject to scarring) such as those giving rise to corneal cicatrisation; wounds subject to capsular contraction (which is common surrounding breast implants); wounds of blood vessels; wounds of the central and peripheral nervous system (where prevention, reduction or inhibition of scarring may enhance neuronal reconnection and/or neuronal function); wounds of tendons, ligaments or muscle; wounds of the oral cavity, including the lips and palate (for example, to inhibit scarring resulting from treatment of cleft lip or palate); wounds of the internal organs such as the liver, heart, brain, digestive tissues and reproductive tissues; wounds of body cavities such as the abdominal cavity, pelvic cavity and thoracic cavity (where inhibition of scarring may reduce the number of incidences of adhesion formation and/or the size of adhesions formed); and surgical wounds (in particular wounds associated with cosmetic procedures, such as scar revision). It is particularly preferred that the medicaments and methods of the invention regarding wounds be used to promote healing of wounds of the skin.

[0088] A medicament in accordance with this aspect of the invention may be formulated in any appropriate carrier. Suitable carriers are pharmaceutically acceptable carriers, for example, preferably those consistent with administration topically or administration by injection for treating wounds and treating or preventing fibrosis; preferably those consistent with administration intravenously or administration by injection or cannulation for treating or preventing metastasis. It will be appreciated that, while the inhibitor of Cep192 or CEP192 may be administered by the same route and in the same form in each incidence of treatment, different incidences of treatment may provide the inhibitor of Cep192 by different medicaments and/or different routes of administration. In embodiments of the invention the initial incidence of treatment may provide the inhibitor of Cep192 by means of an injection, such as an intradermal injection, while the second (and any subsequent) incidences of treatment may involve provision of the inhibitor of Cep192 by alternative routes, such as topical formulations, or vice versa. In an embodiment, multiple administrations of the inhibitor of Cep192 may be effected by the same means or route.

[0089] In an embodiment the shRNA or siRNA inhibitor of CEP192 or of Cep192 gene product expression can be administered such that it is transfected into one or more cells.

[0090] In a preferred embodiment, the inhibitor is an siRNA. In a preferred embodiment the siRNA is encapsulated in a nanoparticle. In an embodiment, the nanoparticle comprises a hydrogel/sugar glass composite. In an embodiment the nanoparticle is a liposomal nanoparticle. In an embodiment, the nanoparticle is PEGylated. In embodiments the PEG is PEG-500 or PEG-3000 or PEG-5000. In an embodiment, the nanoparticle is doped with amino silanes. In an embodiment, the nanoparticle is paramagnetic.

[0091] In a non-limiting embodiment the inhibitor of CEP192 or of Cep192 gene product is provided in a bulk-eroding system such as polylactic acid and glycolic acid (PLGA) copolymer based microspheres or microcapsules systems containing the inhibitor of Cep192. In an embodiment, blends of PLGA:ethylcellulose systems may be used as an appropriate carrier. A further medicament in accordance with this aspect of the invention may be formulated in a surface-eroding system wherein the inhibitor of Cep192 is embedded in an erodible matrix such as the poly(ortho) ester and polyanhydride matrices wherein the hydrolysis of the polymer is rapid. A medicament in accordance with this aspect of the invention may also be formulated by combining a pulsatile delivery system as described above and an immediate release system such as a lyophilized injectable composition described above.

[0092] The inhibitor may be used in a composition with additives. Examples of suitable additives are sodium alginate, as a gelatinizing agent for preparing a suitable base, or cellulose derivatives, such as guar or xanthan gum, inorganic gelatinizing agents, such as aluminum hydroxide or bentonites (termed thixotropic gel-formers), polyacrylic acid derivatives, such as Carbopol®, polyvinylpyrrolidone, microcrystalline cellulose and carboxymethylcellulose. Amphiphilic low molecular weight and higher molecular weight compounds, and also phospholipids, are also suitable. The gels can be present either as water-based hydrogels or as hydrophobic organogels, for example based on mixtures of low and high molecular weight paraffin hydrocarbons and vaseline. The hydrophilic organogels can be prepared, for example, on the basis of high molecular weight polyethylene glycols. These gelatinous forms are washable. Hydrophobic organogels are also suitable. Hydrophobic additives, such as petroleum jelly, wax, oleyl alcohol, propylene glycol monostearate and/or propylene glycol monopalmitostearate, in particular isopropyl

myristate can be included. In an embodiment the inhibitor is in a composition comprising one or more dyes, for example yellow and/or red iron oxide and/or titanium dioxide for the purpose of matching as regards color. Compositions may be in any suitable form including gels, lotions, balms, pastes, sprays, powders, bandages, wound dressing, emulsions, creams and ointments of the mixed-phase or amphiphilic emulsion systems (oil/water-water/oil mixed phase), liposomes and transfersomes or plasters/band aid-type coverings. Emulsifiers which can be employed in compositions comprising the inhibitor of CEP192 or of Cep192 include anionic, cationic or neutral surfactants, for example alkali metal soaps, metal soaps, amine soaps, sulphurated and sulphonated compounds, invert soaps, higher fatty alcohols, partial fatty acid esters of sorbitan and polyoxyethylene sorbitan, e.g. lanette types, wool wax, lanolin or other synthetic products for preparing the oil/water and/or water/oil emulsions.

[0093] Compositions comprising the inhibitor ofCEP192 or of Cep192 can also comprise vaseline, natural or synthetic waxes, fatty acids, fatty alcohols, fatty acid esters, for example as monoglycerides, diglycerides or triglycerides, paraffin oil or vegetable oils, hydrogenated castor oil or coconut oil, hog fat, synthetic fats (for example based on caprylic acid, capric acid, lauric acid or stearic acid, such as Softisan®), or triglyceride mixtures, such as Miglyol®, can be used as lipids, in the form of fatty and/or oleaginous and/or waxy components for preparing the ointments, creams or emulsions of the compositions comprising the inhibitor of CEP192 or of Cep192 used in the methods described herein.

[0094] Osmotically active acids and alkaline solutions, for example hydrochloric acid, citric acid, sodium hydroxide solution, potassium hydroxide solution, sodium hydrogen carbonate, may also be ingredients of the compositions of the invention and, in addition, buffer systems, such as citrate, phosphate, tris buffer or triethanolamine, for adjusting the pH. It is possible to add preservatives as well, such as methyl benzoate or propyl benzoate (parabens) or sorbic acid, for increasing the stability.

[0095] Pastes, powders and solutions are additional forms of compositions comprising the inhibitor of Cep192 which can be applied topically. As consistency-imparting bases, the pastes frequently contain hydrophobic and hydrophilic auxiliary substances, preferably, however, hydrophobic auxiliary substances containing a very high proportion of solids. In order to increase dispersity, and also flowability and slipperiness, and also to prevent agglomerates, the powders or topically applicable powders can, for example, contain starch

species, such as wheat or rice starch, flame-dispersed silicon dioxide or siliceous earth, which also serve as diluent.

[0096] In an embodiment, insofar as the methods herein pertain to wounds or scarring, the compositions comprise further active ingredients suitable for protecting or aiding in healing of the wound, for example one or more antibiotics, antiseptics, vitamins, anesthetics, antihistamines, anti-inflammatory agents, moisturizers, penetration-enhancing agents and/or anti-irritants.

[0097] In an embodiment of the methods and compositions described herein the subject is a mammal. In an embodiment the subject is human.

[0098] As used herein, “promotion” of wound healing, or grammatical equivalent, means an acceleration in any one or more of visual appearance of wound recovery, reduction in wound size, reduction in distance between wound margins, scab formation, fibroplasia and re-epithelialization as compared to the corresponding parameter in an untreated wound.

[0099] As used herein, "wound" is a break or discontinuity in the structure of an organ or tissue (including skin), which includes epithelium, connective tissue, and muscle tissue, caused by an external agent. Examples of wounds include, but are not limited to, skin wounds, ulcerations, bedsores, grazes, tears, cuts, punctures, tympanic membrane perforations, burns, and those that are a consequence of plastic surgery procedures.

[00100] A method is provided for identifying a candidate agent for treating a wound comprising:

a) determining the activity of an amount of Cep192 gene product; and

b) contacting the amount of Cep192 gene product with the candidate agent and determining the activity of the amount of Cep192 gene product in the presence of the candidate agent,

wherein a decreased activity of the amount of Cep192 gene product in the presence of the candidate agent as compared to the activity of Cep192 gene product in the absence of the candidate agent indicates that the candidate agent can treat a wound, and wherein no change in or an increased activity of the amount of Cep192 gene product in the presence of the candidate agent as compared to the activity of Cep192 gene product in the absence of the candidate agent does not indicate that the candidate agent can treat a wound. In an embodiment, the candidate agent is a small molecule of 2000 Daltons or less. In an

embodiment, the candidate agent is a small molecule of 1000 Daltons or less. In an embodiment, the candidate agent is a small molecule of 1500 Daltons or less. In an embodiment, the candidate agent is a substituted or un-substituted hydrocarbon small molecule. In an embodiment, the inhibitor or the candidate agent is an aptamer, a nucleic acid, an oligonucleotide, or a small organic molecule of 2000 Daltons or less. In an embodiment, the inhibitor is cell-membrane permeable.

[00101] With regard to the methods described herein to identify candidate agents as inhibitors of Kif19 or of KIF19 of of CEP192 or of Cep192, one skilled in the art can readily screen libraries of compounds, for example small molecule libraries, using the methods as described to identify agents which are inhibitors of Kif19 or of KIF19 of of CEP192 or of Cep192 and which are therapeutic in treating wounds and promoting the healing of wounds. In addition, one skilled in the art can employ the method to identify peptides, peptidomimetics, antibodies, antibody fragments and nucleic acids which are inhibitors of Kif19 or of KIF19 of of CEP192 or of Cep192 and which are therapeutic in treating wounds and promoting the healing of wounds.

[00102] An inhibitor of KIF19, or of Kif19 gene product is provided for treating metastasis or inhibiting metastasis in a subject having a cancer.

[00103] An inhibitor of CEP192 or of Cep192 gene product is provided for treating metastasis or inhibiting metastasis in a subject having a cancer.

[00104] An inhibitor of KIF19, or of Kif19 gene product, is provided for treating fibrosis or scarring in a subject in need thereof.

[00105] An inhibitor of CEP192 or of Cep192 gene product, is provided for treating fibrosis or scarring in a subject in need thereof.

[00106] An inhibitor of CEP192 or of Cep192 gene product, for treating pain associated with wound healing in a subject.

[00107] In an embodiment, the inhibitor is an RNAi nucleic acid. In an embodiment, the inhibitor comprises an siRNA. In an embodiment, the inhibitor comprises an shRNA. In an embodiment, the siRNA or shRNA is directed against CEP192. In an embodiment, the siRNA or shRNA is directed against KIF19.

[00108] In an embodiment of the methods, prodcuts and compositions, the inhibitor is biomembrane-permeable or is conjugated or otherwise attached to a moiety which renders the inhibitor biomembrane-permeable.

[00109] In an embodiment, KIF19 comprises the following sequence (SEQ ID NO:1):

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   1 gcgttgttgg tttcgggttg tcaggcagcg cgcgaggcgg cgggcagcta gcagctggcg
  61 gacgcgaccc ggaggcggtg ggggtgcggc tgagccatgc ccggtggcgc ggcctgagcc
 121 cctccacctg ctgcaatcat gaaggacagc ggggactcca aggaccagca actcatggtg
 181 gcgcttcggg tccggcccat cagcgtggca gagctggagg aaggagctac cctcatcgcc
 241 cataaagtgg atgagcagat ggtggttctc atggacccaa tggaggatcc cgacgacatc
 301 ctgcgggcgc atcgctcccg ggagaagtcc tacctgttcg acgtggcctt tgacttcacc
 361 gccacccagg agatggtgta tcaggccacc accaagagcc tcatcgaggg cgtcatctca
 421 ggctacaatg ccactgtctt tgcctatggc cccacaggct gtgggaaaac ctacaccatg
 481 ctgggcacag accaggagcc tggcatctat gttcagaccc tcaacgacct cttccgtgcc
 541 atcgaggaga ccagcaatga catggagtat gaggtctcca tgtcctacct ggagatctac
 601 aatgagatga tccgggacct gctgaacccc tccctgggct acctggagct gcgggaggac
 661 tctaaggggg tgatccaggt ggccggcatc accgaagtct ccaccatcaa tgccaaggag
 721 atcatgcagc tgctgatgaa ggggaaccgg cagaggaccc aggagcccac ggccgccaac
 781 cagacgtcct cccgctccca cgcggtactg caggtgaccg tgcgccagcg cagccgggtc
 841 aagaacatct tgcaggaggt gcggcagggc cgcctgttca tgatcgacct ggctggctca
 901 gagcgcgcct cgcagacaca gaatcgtggg cagcgtatga aggagggggc ccacatcaac
 961 cgctcactgc tggcactggg caactgcatc aacgccctga gcgacaaggg tagcaacaag
1021 tacatcaact atcgcgacag caagctcacc cggctcctga aggactctct gggaggaaac
1081 agccgcacag tgatgatcgc tcacatcagt cctgcgagca gtgccttcga ggagtcccgg
1141 aacaccctga cctacgccgg ccgggccaag aacattaaga ctagggtgaa gcagaacctc
1201 ctgaacgtct cctaccacat cgcccagtac accagcatca tcgctgacct gcggggcgag
1261 atccagcgac tcaagcgcaa gattgatgag cagactgggc ggggccaggc ccggggccgg
1321 caggatcggg gtgacatccg ccacatccaa gctgaggtcc agctgcacag cgggcagggt
1381 gagaaggctg gcatgggaca gcttcgggag cagctcgcca gcgccttcca ggagcagatg
1441 gatgtgcgga ggcgcctgct ggagctggag aaccgcgcca tggaggtcca gattgacacc
1501 tcccgacacc tgctcaccat cgccggctgg aagcatgaga agtcccgccg ggccctcaaa
1561 tggcgggagg agcagcgaaa ggagtgctac gctaaggacg acagcgagaa ggactcagac
1621 acaggtgatg accaaccaga catcctggag ccacccgagg tggccgcagc ccgggagagc
1681 attgcagccc tggtggacga gcagaagcaa ctgcgcaagc agaagctggc gctggagcag
1741 cgctgccggg agctgcgcgc gcggggccgg cgcctggagg agacgctgcc gcggcgcatc
1801 ggctccgagg agcagcgcga ggtgctcagc ctgctgtgcc gcgtgcacga gctcgaggtg
1861 gagaacaccg agatgcagtc gcacgcgctg ctccgcgacg gtgcgctccg ccaccgccac
1921 gaggccgtgc gccgcctgga gcagcaccgc agtctctgcg acgagattat ccagggccag
1981 cggcagatca tcgacgacta caacctggcc gtcccgcagc gcctggaaga gctctacgaa
2041 gtgtacctgc gggagctgga ggagggcagc ctggagcagg ccaccatcat ggaccaagtg
2101 gcctccaggg ccctgcagga cagctccttg cccaaaatta ccccagcagg aacctcactg
2161 accccagatt ctgacctgga gagtgtgaag acattgagct ctgatgccca gcacctgcag
2221 aacagcgccc tccctccccc cagcacagag agtgaaggcc accacgtgtt caaggctggt
2281 actggggcct ggcaggcaaa aagctcctct gtgcccaccc cacctcccat ccagctcggc
2341 agcctggtga cgcaggaggc cccggctcag gacagcctgg gcagctggat caactcttcc
2401 cctgacagca gtgagaacct gtcggagatc cccttgtccc acaaagagag gaaggagatc
2461 ctgactggca ccaagtgcat ctgggtgaag gccgcccggc ggcgctcgcg ggccctggga
2521 accgaggggc gacacctgct ggcacccgcg acagagcgca gcagcctgtc cctgcactca
2581 ctgagcgagg gcgacgatgc gcggccacca ggcccactgg cctgcaagcg gccgcccagc
2641 cccacactac agcatgctgc cagtgaggac aacctgtcca gcagcacggg cgaggccccg
2701 tcccgggcag tcggacatca tggggacggc cccaggccct ggctgcgtgg ccagaagaaa
2761 agcctgggca agaaaaggga ggagtcgctg gaggcaaaga gaaggaagcg gaggtcccga
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2821 tccttcgagg tcaccgggca agggctctcc caccccaaga cacacctcct ggggccccat
2881 caggcggagc gcatctcgga ccacaggatg ccagtgtgca ggcacccagc ccctggtatc
2941 cggcatctgg gaaaggtcac gctacctttg gccaaagtca aactccctcc aagccagaac
3001 acgggcccgg gggactcctc acccctggct gttcccccca acccaggtgg tggttctcga
3061 cgggctaccc gtgggccccg cctgccccac ggcacaagca cccatggcaa agatggatgc
3121 tcccggcata actgaggggc cctgcctgga actggctctc tcacctccca agactgaatg
3181 gggtctagca gggcatggga ggtggaggct gggcagatgg agatgaccag gaagtaagct
3241 caggatctca gcaggccagg gctcctgaga cccaggaact ggggtctctg cccaaccctc
3301 ccatgctttc agtgccactg gggaaaagag gtgaggccag gggacatggc caggacggct
3361 gggctccctg gcttcccagc cctggacaga atgctgttgc caaaacctgc acagccctga
3421 ggccagcctc ggccttggta acggaggaaa gcagctgaca gtgagacggg gctcctggcc
3481 cacgtgtggg gcacgggcat cctggatggt tggggaggcg ccgacaggca cttcacgtat
3541 tacaattggg gatgtgggtg agggagggaa tctggttttg ttacttggca gtggtttttt
3601 ctcacccttc ctttttaaca ataaaatccc atttgggtct tgaaaaaaaa aaaaaaaaaa
3661 aaaaaaaaaa

[00110] In an embodiment, Kif19 gene product comprises the following sequence (SEQ ID NO:2):

MKDSGDSKDQQLMVALRVRPISVAELEEGATLIAHKVDEQMVVL
MDPMEDPDDILRAHRSREKSYLFDVAFDFTATQEMVYQATTKSLIEGVISGYNATV
FAYGPTGCGKTYTMLGTDQEPGIYVQTLNDLFRAIEETSNDMEYEVSMSYLEIYNE
MIRDLLNPSLGYLELREDSKGVIQVAGITEVSTINAKEIMQLLMKGNRQRTQEPTAA
NQTSSRSHAVLQVTVRQRSRVKNILQEVRQGRLFMIDLAGSERASQTQNRGQRMK
EGAHINRSLLALGNCINALSDKGSNKYINYRDSKLTRLLKDSLGGNSRTVMIAHISP
ASSAFEESRNTLTYAGRAKNIKTRVKQNLLNVSYHIAQYTSIIADLRGEIQRLKRKID
EQTGRGQARGRQDRGDIRHIQAEVQLHSGQGEKAGMGQLREQLASAFQEQMDVR
RRLLELENRAMEVQIDTSRHLLTIAGWKHEKSRRALKWREEQRKECYAKDDSEKD
SDTGDDQPDILEPPEVAAARESIAALVDEQKQLRKQKLALEQRCRELRARGRRLEE
TLPRRIGSEEQREVLSLLCRVHELEVENTEMQSHALLRDGALRHRHEAVRRLEQHR
SLCDEIIQGQRQIIDDYNLAVPQRLEELYEVYLRELEEGSLEQATIMDQVASRALQD
SSLPKITPAGTSLTPDSDLESVKTLSSDAQHLQNSALPPLSTESEGHHVFKAGTGAW
QAKSSSVPTPPPIQLGSLVTQEAPAQDSLGSWINSSPDSSENLSEIPLSHKERKEILTG
TKCIWVKAARRRSRALGTEGRHLLAPATERSSLSLHSLSEGDDARPPGPLACKRPPS
PTLQHAASEDNLSSSTGEAPSRAVGHHGDGPRPWLRGQKKSLGKKREESLEAKRR
KRRSRSFEVTGQGLSHPKTHLLGPHQAERISDHRMPVCRHPAPGIRHLGKVTLPLA
KVKLPPSQNTGPGDSSPLAVPPNPGGGSRRATRGPRLPHGTSTHGKDGCSRHN

[00111] In an embodiment, CEP192 comprises the following sequence (SEQ ID NO:3):

1 agtgccctgg gacacctctt cagtccgtgg actttcccgc tgcacactgc cctccgaagt
61 cggggacgcg ggctcgtgag atggaagatt ttcgaggtat agcagaagaa tcatttccaa
121 gctttctcac caattcatta tttggtaaca gtgggatttt ggaaaatgtc actctttctt
181 caaatcttgg cttgcctgtt gctgtttcta cacttgctag ggatagatcc agcactgata
241 acaggtatcc tgatatccag gcatcttact tagtagaagg gagattttca gttccatccg
301 ggtcatctcc cggaagccag agtgatgctg aaccaagaga gaggttacag cttagcttcc
361 aggatgatga ttctatctct aggaaaaaga gctatgtgga aagtcaacgt ttgtcaaatg

421 ctctcagcaa acagtcagct ttacaaatgg agacagcagg accagaagag gagccagccg
481 gagctacaga atccttgcag ggccaagatc tcttcaacag ggcttcacca ctggaacaag
541 cacaagactc acctattgat tttcatttac agtcatggat gaataataag gaacccaaga
601 ttgttgtgct tgatgctgga aaacattttg aagacaagac tctaaagagt gacctaagcc
661 acactagctt attagaaaat gagaaactta tcttaccgac aagcttggaa gattcttctg
721 atgatgatat tgatgatgaa atgttttatg atgatcattt ggaggcttat tttgaacaac
781 tggcaattcc aggaatgata tatgaagacc tagaaggacc agaacctcca gaaaaaggtt
841 ttaagttacc tacaaatggt cttagacagg caaatgaaaa cggtagctta aactgcaagt
901 ttcaatcaga aaataacagc tctctgattt ccctcgactc acactcttct gaaacaactc
961 acaaagagtc tgaggaaagc caagttattt gtctacctgg gactagtaat tctataggta
1021 ctggagatag tagaaggtac acagatggta tgttaccatt ttcctctggt acttggggaa
1081 ctgagaaaga aatagaaaat ttgaagggta ttgttccaga tcttaacagt gaatgtgcaa
1141 gtaaagatgt tctggtgaag accctcaggg ctattgatgt gaaacttaac tctgataatt
1201 ttcatgatgc aaatgccaat agaggtggtt ttgatctgac tgaccctgta aaacaggggg
1261 cagagtgtcc tcaccaaaat aagacagttt tgcacatgga tggatgttta gacactgaga
1321 ctcctacggt gtccattcaa gaaaatgtgg atgtagcctc tttgaagccc attagtgaca
1381 gtggaattaa tttcactgat gccatttggt caccaacttg tgaaaggcga acatgtgaat
1441 gtcacgagtc catcgaaaag aataaagaca aaacagatct cccacagagt gtggtctatc
1501 aaaatgaaga gggtaggtgg gtcacagacc ttgcctatta cacatctttt aatagcaaac
1561 aaaatttaaa tgtgtctcta agtgatgaga tgaatgaaga cttcagatct ggttctgaag
1621 catttgattt gattgcacaa gatgaagaag aatttaataa agagcatcaa tttatacagg
1681 aagaaaacat agatgctcat aatacttcgg ttgcactggg cgatacgtcc tggggagcta
1741 caattaatta cagtctgttg aggaaatcac gtagcacatc agatttggat aaagatgatg
1801 ccagttattt acgtctgtct ttaggagagt tctttgctca aagatctgaa gctcttggtt
1861 gccttggtgg tggtaacaat gtgaaaagac catcatttgg ctattttatt agatcaccag
1921 agaagagaga acctattgcc ttaataagaa aatctgatgt atcaagaggt aatttggaaa
1981 aagaaatggc tcatcttaac catgatctat attcaggaga tttaaatgaa cagtcccagg
2041 cacagctaag tgaaggatca attacacttc aggttgaagc agtagagagt acttcacaag
2101 tggatgaaaa tgatgtgacg ttaacggctg ataaaggcaa aacagaggac actttcttca
2161 tgagcaacaa accccaaaga tacaaagaca agctaccaga tagtggtgat tctatgctta
2221 ggatcagcac cattgcttca gccattgcag aggcatcagt taatactgat ccttcccaac
2281 ttgctgcaat gatcaaggca ctttcaaata aaaccagaga caagactttt caggaagatg
2341 agaaacaaaa ggactattct catgtgcgtc atttcttacc taatgattta gaaaaaagta
2401 atggatccaa tgcacttgat atggagaaat accttaaaaa aacagaagtt agtagatatg
2461 aaagtgcatt ggaaaacttt tcaagggcta gtatgtctga tacttgggat ttatctttgc
2521 ccaaagaaca aactactcaa gacattcatc cggtggactt aagtgctact agtgtaagtg
2581 tgagggcacc agaagaaaac acagcagcta ttgtttatgt tgaaaatgga gagagtgaga
2641 atcaagagtc atttagaacc ataaactcct caaattcagt tacaaataga gagaataaca
2701 gtgcagtagt tgatgtgaag acatgttcca ttgacaacaa attacaagat gttggtaacg
2761 atgaaaaagc tacctcaatt tccactccat ctgatagtta ttcatcagtg aggaacccca
2821 gaataacatc cctttgtctg ttaaaagact gtgaagaaat acgagataac agagaaaatc
2881 agaggcaaaa tgagtgtgtc agtgaaataa gcaacagtga gaagcatgtg acttttgaaa
2941 accatcgcat agtctcacct aaaaatagtg atttgaaaaa tacctctcct gagcatggtg

3001 gacgtggctc agaggatgag caggagagct tcagaccttc cacgtcacca ctgagtcatt
3061 cttctcctag tgaaatttct ggaacgagtt catcagggtg tgcgttagag tcctttggtt
3121 cagcagctca gcagcagcag cctccctgtg agcaggagtt gtctcccttg gtgtgctcgc
3181 ctgctggggt gagcaggctg acgtatgtgt ctgaaccaga gagctcctat cctaccacag
3241 ccacagatga tgccctggag gaccgcaaga gtgatatcac cagcgagttg agtaccacaa
3301 ttattcaagg cagtccagcc gcattggagg aacgggctat ggaaaaattg agagaaaaag
3361 ttccatttca gaatagagga aaaggaacat tatcatctat tatccagaat aactctgata
3421 caagaaaagc aactgaaact acttctctga gtagcaagcc tgaatatgta aaacctgact
3481 ttagatggag taaagatcct tcctccaaaa gtggaaatct gttggaaacc agtgaggtag
3541 gttggacatc aaaccctgag gaattggacc cgatcaggct ggctctcctg ggcaagtcag
3601 gtctgagctg tcaggtgggg tcagccacat cacaccctgt gtcctgccag gagcctatag
3661 atgaagatca aagaataagt cctaaagata agtcaactgc tggccgtgag ttcagtggcc
3721 aggtttctca tcagaccacc tctgaaaacc agtgtactcc tattcccagc agcacagttc
3781 acagctctgt ggctgacatg cagaacatgc ctgctgctgt gcacgcactc ttgacacaac
3841 cctctctcag cgctgctcct tttgctcagc ggtatttggg aacactccct tcaactggaa
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4261 tcagtgtgct taatccaact gaccgctggc tgcaagtcag cattggggtc ctcagcatta
4321 gtgttaatgg tgaaaaggtg gatctttcaa catatcgttg tttagttttc aagaataaag
4381 ccatcataag acctcatgcc acagaagaga taaaagtgct ttttatacca tccagtcctg
4441 gggttttcag atgcacattc agtgttgctt cttggccatg ttcgacagat gctgagacca
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4561 ttattgaggt agaaacagaa aagaaagacg ttcttgattt tggtgacttg acttatggag
4621 gctggaaagc cctcccacta aaattgataa accgaacgca tgccactgtg ccaattagac
4681 tgattattaa tgctaacgct gtagcctggc gctgtttcac gttttccaag gaatccgtcc
4741 gagctcctgt ggaagttgct ccttgcgctg atgtggtcac tcggctagca ggcccttctg
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4861 gggttctttt ccatagtcca aagaaacaga tcagctcttc agatattctg gactcagcag
4921 aagaattctc ggcaaaagtt gatatcgaag ttgacagccc aaaccctacg cccgttctta
4981 gaagtgtgag tctccgagca agagcaggaa tagctaggat ccatgctccc agggacttgc
5041 agacgatgca tttcttggcc aaagtggctt cctcaagaaa gcagcactta cctttgaaaa
5101 atgctgggaa cattgaagtt tatttggata tcaaggtccc agaacaagga agtcactttt
5161 cagtggatcc aaagaatcta ctccttaaac ctggagaaga acatgaggtt attgtttcat
5221 ttactccaaa ggatcctgaa gcctgcgagg aaaggatctt gaaaatattt gtgcagccat
5281 ttggacctca gtatgaggta gtgttaaaag gcgaagtcat ttcttcagga agtaaacctc
5341 tgtcacctgg accttgctta gatattccat cgattttgtc caacaaacaa tttctggctt
5401 ggggaggagt ccctctaggt agaacacagc ttcagaaact agctttaaga aataattctg
5461 catctacaac tcaacattta cgactgctta ttagaggaca agatcaggac tgctttcagc
5521 ttcagaacac ttttggttca gaacagcgat tgaccagtaa ctgtgagatc agaattcacc

5581 caaaggaaga cattttcatc tctgtattat ttgcacctac tcgattatct tgcatgttgg
5641 ctagactaga aatcaaacaa cttggaaatc gatcacaacc aggcattaag ttcacaatac
5701 ctttgtctgg atatggagga acaagcaatc ttattttgga aggcgttaaa aaattatctg
5761 acagttacat ggtaacagtg aatggcttag tacctggcaa agaaagtaaa attgtttttt
5821 ctgtccgcaa cactggctcc cgagcagctt ttgttaaagc agtaggtttt aaggattctc
5881 agaaaaaagt tttgctggat cctaaagtat tgaggatttt tccagataaa tttgtactca
5941 aggaaagaac acaagaaaat gttactttaa tatataatcc atcagacaga ggaatcaata
6001 ataaaactgc aacagaacta tcaactgtat acttatttgg tggagatgaa atttcaagac
6061 agcagtatcg cagggccctg ttacataaac cagagatgat aaaacagata cttccagaac
6121 atagtgtgct tcaaaacatt aattttgttg aagcatttca agatgagcta ttagtaactg
6181 aagtatatga tcttccccaa cgacctaatg atgttcagct cttttatgga agcatgtgta
6241 aaattatact ttcagtaatt ggagaattca gagattgcat ttctagcaga gaattccttc
6301 agccttcttc caaagctagc ttggaatcta caagcgactt gggagcttct gggaaacatg
6361 gtggcaacgt ctctttggat gttttaccag tcaaaggtcc tcagggttct cctcttctct
6421 cacgggcggc tcgcccgcct ctggatcagc tggcctccga agagccgtgg actgtcctac
6481 ccgagcactt gattctggta gctccttctc cttgtgacat ggcaaaaact ggacgtttcc
6541 agattgtgaa taactctgtg aggttactga gatttgagct gtgctggcca gcgcattgcc
6601 tcacagtcac gccgcagcat ggatgtgtcg cgccagagag taaactacaa attcttgtga
6661 gtcctaattc ctccttatcc acaaaacagt caatgttccc gtggagtggt ttgatctata
6721 tacactgtga cgatggacag aagaaaattg tgaaagttca aattcgagaa gatttaactc
6781 aagtggaact tttaactcgt ttgacctcca aaccatttgg aattctttcc ccagtatctg
6841 agccttcagt tagtcatttg gtcaaaccaa tgacaaaacc gccttccaca aaagttgaaa
6901 taagaaacaa gagtattact tttcctacaa cagaacctgg tgaaacttca gagagctgtc
6961 tagaactcga gaatcatggc accacagacg tgaaatggca tctgtcatct ttagcgccac
7021 cttatgtcaa gggagttgat gaaagtggag atgtttttag agctacctat gcagcattca
7081 gatgttctcc tatttctggt ctgctggaaa gccatgggat ccaaaaagtc tccatcacat
7141 ttttgcccag aggtaggggg gattatgccc agttttggga tgttgaatgt caccctctta
7201 aggagcctca catgaaacac acgttgagat tccaactctc tggacaaagc atcgaagcag
7261 aaaatgagcc tgaaaacgca tgcctttcca cggattccct cattaaaata gatcatttag
7321 ttaagccccg aagacaagct gtgtcagagg cttctgctcg catacctgag cagcttgatg
7381 tgactgctcg tggagtttat gccccagagg atgtgtacag gttccggccg actagtgtgg
7441 gggaatcacg gacacttaaa gtcaatctgc gaaataattc ttttattaca cactcactga
7501 agtttttgag tcccagagag ccattctatg tcaaacattc caagtactct ttgagagccc
7561 agcattacat caacatgccc gtgcagttca aaccgaagtc cgcaggcaaa tttgaagctt
7621 tgcttgtcat tcaaacagat gaaggcaaga gtattgctat tcgactaatt ggtgaagctc
7681 ttggaaaaaa ttaactagaa tacatttttg tgtaaagtaa attacataag ttgtattttg
7741 ttaactttat ctttctacac tacaattatg cttttgtata tatattttgt atgatggata
7801 tctataattg tagattttgt ttttacaagc taatactgaa gactcgactg aaatattatg
7861 tatctagccc atagtattgt acttaacttt tacaggtgag aagagagttc tgtgtttgca
7921 ttgattatga tattctgaat aaatatggaa tatattttaa tgtggtatat ccagaaaaaa
7981 aaaaaaaaaa aaaaa

[00112] In an embodiment, Cep192 gene product comprises the following sequence (SEQ ID NO:4):

MEDFRGIAEESFPSFLTNSLFGNSGILENVTLSSNLGLPVAVST
LARDRSSTDNRYPDIQASYLVEGRFSVPSGSSPGSQSDAEPRERLQLSFQDDDSISRK
KSYVESQRLSNALSKQSALQMETAGPEEEPAGATESLQGQDLFNRASPLEQAQDSPID
FHLQSWMNNKEPKIVVLDAGKHFEDKTLKSDLSHTSLLENEKLILPTSLEDSSDDDID
DEMFYDDHLEAYFEQLAIPGMIYEDLEGPEPPEKGFKLPTNGLRQANENGSLNCKFQS
ENNSSLISLDSHSSETTHKESEESQVICLPGTSNSIGTGDSRRYTDGMLPFSSGTWGT
EKEIENLKGIVPDLNSECASKDVLVKTLRAIDVKLNSDNFHDANANRGGFDLTDPVKQ
GAECPHQNKTVLHMDGCLDTETPTVSIQENVDVASLKPISDSGINFTDAIWSPTCERR
TCECHESIEKNKDKTDLPQSVVYQNEEGRWVTDLAYYTSFNSKQNLNVSLSDEMNEDF
RSGSEAFDLIAQDEEEFNKEHQFIQEENIDAHNTSVALGDTSWGATINYSLLRKSRST
SDLDKDDASYLRLSLGEFFAQRSEALGCLGGGNNVKRPSFGYFIRSPEKREPIALIRK
SDVSRGNLEKEMAHLNHDLYSGDLNEQSQAQLSEGSITLQVEAVESTSQVDENDVTLT
ADKGKTEDTFFMSNKPQRYKDKLPDSGDSMLRISTIASAIAEASVNTDPSQLAAMIKA
LSNKTRDKTFQEDEKQKDYSHVRHFLPNDLEKSNGSNALDMEKYLKKTEVSRYESALE
NFSRASMSDTWDLSLPKEQTTQDIHPVDLSATSVSVRAPEENTAAIVYVENGESENQE
SFRTINSSNSVTNRENNSAVVDVKTCSIDNKLQDVGNDEKATSISTPSDSYSSVRNPR
ITSLCLLKDCEEIRDNRENQRQNECVSEISNSEKHVTFENHRIVSPKNSDLKNTSPEH
GGRGSEDEQESFRPSTSPLSHSSPSEISGTSSSGCALESFGSAAQQQQPPCEQELSPL
VCSPAGVSRLTYVSEPESSYPTTATDDALEDRKSDITSELSTTIIQGSPAALEERAME
KLREKVPFQNRGKGTLSSIIQNNSDTRKATETTSLSSKPEYVKPDFRWSKDPSSKSGN
LLETSEVGWTSNPEELDPIRLALLGKSGLSCQVGSATSHPVSCQEPIDEDQRISPKDK
STAGREFSGQVSHQTTSENQCTPIPSSTVHSSVADMQNMPAAVHALLTQPSLSAAPFA
QRYLGTLPSTGSTTLPQCHAGNATVCGFSGGLPYPAVAGEPVQNSVAVGICLGSNIGS
GWMGTSSLCNPYSNTLNQNLLSTTKPFPVPSVGTNCGIEPWDSGVTSGLGSVRVPEEL
KLPHACCVGIASQTLLSVLNPTDRWLQVSIGVLSISVNGEKVDLSTYRCLVFKNKAII
RPHATEEIKVLFIPSSPGVFRCTFSVASWPCSTDAETIVQAEALASTVTLTAIAESPV
IEVETEKKDVLDFGDLTYGGWKALPLKLINRTHATVPIRLIINANAVAWRCFTFSKES
VRAPVEVAPCADVVTRLAGPSVVNHMMPASYDGQDPEFLMIWVLFHSPKKQISSSDIL
DSAEEFSAKVDIEVDSPNPTPVLRSVSLRARAGIARIHAPRDLQTMHFLAKVASSRKQ
HLPLKNAGNIEVYLDIKVPEQGSHFSVDPKNLLLKPGEEHEVIVSFTPKDPEACEERI
LKIFVQPFGPQYEVVLKGEVISSGSKPLSPGPCLDIPSILSNKQFLAWGGVPLGRTQL
QKLALRNNSASTTQHLRLLIRGQDQDCFQLQNTFGSEQRLTSNCEIRIHPKEDIFISV
LFAPTRLSCMLARLEIKQLGNRSQPGIKFTIPLSGYGGTSNLILEGVKKLSDSYMVTV
NGLVPGKESKIVFSVRNTGSRAAFVKAVGFKDSQKKVLLDPKVLRIFPDKFVLKERTQ
ENVTLIYNPSDRGINNKTATELSTVYLFGGDEISRQQYRRALLHKPEMIKQILPEHSV
LQNINFVEAFQDELLVTEVYDLPQRPNDVQLFYGSMCKIILSVIGEFRDCISSREFLQ
PSSKASLESTSDLGASGKHGGNVSLDVLPVKGPQGSPLLSRAARPPLDQLASEEPWTV
LPEHLILVAPSPCDMAKTGRFQIVNNSVRLLRFELCWPAHCLTVTPQHGCVAPESKLQ
ILVSPNSSLSTKQSMFPWSGLIYIHCDDGQKKIVKVQIREDLTQVELLTRLTSKPFGI
LSPVSEPSVSHLVKPMTKPPSTKVEIRNKSITFPTTEPGETSESCLELENHGTTDVKW

HLSSLAPPYVKGVDESGDVFRATYAAFRCSPISGLLESHGIQKVSITFLPRGRGDYAQ
FWDVECHPLKEPHMKHTLRFQLSGQSIEAENEPENACLSTDSLIKIDHLVKPRRQAVS
EASARIPEQLDVTARGVYAPEDVYRFRPTSVGESRTLKVNLRNNSFITHSLKFLSPRE
PFYVKHSKYSLRAQHYINMPVQFKPKSAGKFEALLVIQTDEGKSIAIRLIGEALGKN

[00113] The phrase "and/or" as used herein, with option A and/or option B for example, encompasses the individual embodiments of (i) option A alone, (ii) option B alone, and (iii) option A plus option B.

[00114] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[00115] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group subjectly and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

[00116] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[00117] In the event that one or more of the literature and similar materials incorporated by reference herein differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

[00118] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

Introduction

[00119] Cell motility is driven by a cycle of protrusion of the membrane at the cell front, adhesion of the protrusion to the substratum, contractility to move the cell body forward, and finally disadhesion at the rear. While the roles of the actin cytoskeleton in these events have been studied in detail (Ridley, Schwartz et al. 2003; Gardel, Schneider et al. 2010),

much less is known about the specific contributions of microtubules. It is, however, clear that the microtubule cytoskeleton is required for the normal polarization and motility of many cell types and there is emerging evidence that it does so by exerting spatiotemporal control over actin dynamics/contractility and the delivery of membrane and signaling molecules to the cell periphery (Rodriguez, Schaefer et al. 2003; Small and Kaverina 2003; Watanabe, Noritake et al. 2005). Microtubules also contribute to cell migration by regulating the disassembly of focal adhesions (Broussard, Webb et al. 2008) (FAs). FAs are integrin-based macromolecular assemblies that link the actin cytoskeleton to extracellular matrix and thus anchor the cell to its substratum to provide traction for cell motility. The primary microtubule nucleating and organizing structure in the cell is the centrosome. Herein it is disclosed that Cep192 and Kif19 regulate the microtubule cytoskeleton.

[00120] While it would be difficult to raise the levels of a particular regulatory protein rapidly in relevant cells, it is far more tractable to lower the levels of a target protein through RNA interference (RNAi). The essence of this approach is to inhibit messenger RNA (mRNA) from coding for the synthesis of a target protein. There are various types of RNAi, such as plasmid-driven shRNA, which has the advantage of being targetable to certain cell populations and is generally long-lasting in terms of suppression of protein expression. shRNA generally requires a specialized transfection technique such as viral entry or electroporation. Small interfering RNAs (siRNAs) do not involve the use of a plasmid, are tiny and hence can more readily be introduced into cells, and offer more flexibility in terms of target sequences. siRNA can be handled and treated much like a drug and theoretically can interfere with the translation of almost any mRNA as long as the mRNA has a distinctive sequence. Therefore, siRNA has far broader flexibility than traditional drugs. A key to capitalizing on the therapeutic benefits of siRNA lies in effective delivery systems. Carriers such as nanoparticles have now become the approach of choice. Nanotechnology is broadly considered the study of manipulations of materials at the nanometer scale, roughly 1 to 500 nm. Materials at this scale possess a higher surface to volume ratio and, as a result, their physical properties tend to be different from materials at the macro or micro scale. Novel properties that result from such modifications have led to applications in fields such as catalysis, microelectronics, robotics and medicine. The medical and biological applications are particularly interesting because most biochemical processes, especially those involving macromolecules, occur at the lower end of the nano

scale. Nanotechnology, therefore, holds the promise of being able to duplicate biochemical processes and directly alter these processes using man-made materials. With the progress of material synthesis and the rise of nanotechnology, the generation of nanomaterials with specific functions has become possible. In to, for example, solid tumors in humans.

[00121] To date, the reported liposomal and other nanoparticle based delivery vehicles for siRNA have involved systemic delivery. In contrast, a novel delivery approach disclosed herein is effective for both topical and systemic applications. One preferred embodiment of the platform is based on a hydrogel/sugar glass composite, or hybrid nanoparticle platform capable of encapsulating and controllably releasing a broad range of therapeutically relevant materials ranging from gaseous nitric oxide to peptides to larger macromolecules such as chemotherapeutic agents and phosphodiesterase inhibitors. The versatility of this biocompatible and nontoxic platform has been shown in pre-clinical studies demonstrating: i) topical efficacy in clearing both Gram positive and negative cutaneous wound infections, accelerating wound healing, and promoting erectile activity; and ii) systemic efficacy in modulating cardiovascular parameters.

[00122] The data herein indicates that kif19 and Cep192 proteins exert profound regulatory control over the motility and/or growth characteristics of key cells required for wound closure, re-vascularization and re-innervation. Each protein can be targeted independently by different np-si to control a distinct aspect of the wound healing cascade. The nanoparticle platform can be very effective as a topical delivery vehicle for the siRNA. The preferred therapeutic platform technology is nanoparticle-encapsulated siRNAs (np-si) targeting the expression of CEP192 and KIF19 genes encoding regulators of the microtubule cytoskeleton. Without being bound be theory, it is understood that Kif19 np-si treatments inhibit fibroblast motility to reduce fibrosis/scarring. The Cep192 np-si treatment does similar, but also inhibits axonal growth to ameliorate the pain that results from premature axon sprouting into wounded tissue.

[00123] The nanoparticle delivery system bypasses pitfalls typically associated with therapeutic siRNA - for example, the ability to deliver therapeutic levels of siRNA to enhance the closure of surface wounds *in vivo*.

Example 1

[00124] Kif19 normally promotes cell motility by stimulating the disassembly of integrin-based adhesion complexes that link cells to the underlying extracellular matrix. siRNA-mediated depletion of Kif19 inhibits 1) cancer cell motility *in vitro*; 2) matrigel invasion of primary tumor cells *ex vivo*, 3) and movement of cells into excision wounds in mice.

[00125] Fig. 1 shows a confocal micrograph showing a human U2OS cell double labeled for Kif19 and the FA protein, vinculin. The far right panel is a higher magnification of the region boxed in "merge". As shown in Figs. 2 and 3, the depletion of Kif19 from tissue culture cells induces an increase in focal adhesion size and stability. Fig. 2 shows regions of U2OS cells (human Osteosarcoma) immunostained for the focal adhesion protein vinculin. The depletion of Kif19 by siRNA induces a substantial increase in the size and number of focal adhesions particularly in the cell interior. A time series was obtained showing the assembly/disassembly dynamics of focal adhesions in GFP-vinculin expressing control and Kif19 siRNA-treated U2OS cells. In Fig. 3, panel A shows fluorescence recovery after photobleaching (FRAP) of GFP-vinculin labeled focal adhesions from control and Kif19 siRNA-treated U2OS cells. Panel B shows a representative fluorescence recovery plot from each condition. Panel C plots the density of focal adhesions in untreated cells (pre) and at various time points after nocodazole washout. Repolymerization of MTs after nocodazole washout was previously shown to stimulate focal adhesion disassembly (Ezratty, Partridge et al. 2005). The depletion of Kif19 prevents the disassembly of focal adhesions after nocodazole washout.

[00126] Fig. 4 shows siRNA depletion of Kif19 decreases the motility of cancer cells *in vitro*. Depletion of Kif19 prevents tumor cell invasion from anaplastic thyroid carcinomas embedded in matrigel. Fig. 5 shows an anaplastic thyroid carcinoma mouse model (Archiuch, Rousseau et al, Oncotarget, Dec. 2011). Accounts for 40% of all thyroid cancer deaths and extremely metastatic. Nearly 100% lethality, median survival 4 months. Currently not treatable. Dissociated tumor is removed from mouse and bathed in nanoparticles containing control or Kif19 siRNA for 2-24 hrs. Tumors are then embedded in matrigel and imaged daily. Movement of cells from tumor into matrigel is considered invasion. Black dots moving away from the dark central mass are invasive tumor cells. Kif19 nanoparticle siRNA treatment reduces tumor cell invasion relative to controls.

[00127] Depletion of Kif19 inhibits cell movement into mouse full thickness biopsy wounds. Fig. 6 is images showing the closure of control and Kif-19 siRNA treated full thickness biopsy wounds positioned next to one another on the flank of a mouse. Images were taken 1 and 5 days after wounding. Kif19 siRNA or scrambled RNA (control) were encapsulated in nanoparticles and topically applied to wound.

[00128] Kif-19 depolymerizes microtubules *in vitro*, as shown in Fig. 7 where a time-series of TIRF images shows a field of fluorescently-labeled taxol-stabilized microtubules incubated with purified recombinant full-length Kif19. The time from the first to last image is 5 minutes.

[00129] In summary, Kif19 is a microtubule depolymerizing enzyme *in vitro* that localizes to and stimulates the turnover of substrate adhesions in cells (Fig. 8A-D). siRNA depletion of Kif19 in human epithelial and fibroblast cell models nearly completely suppresses cell motility likely because these cells become too tightly attached to their underlying substratum. Kif19 is the first and, at present, only microtubule regulatory protein known to be housed within the substrate adhesion complex. Agents that suppress Kif19, such as Kif19 siRNA nanoparticles, can be used as a means to prevent fibrosis/scarring later in the wound healing process.

Example 2

[00130] Cep192 promotes cell motility via the nucleation of centrosomal microtubules. Cep192 is a centrosomal scaffolding protein required for the nucleation of microtubules from centrosomes. siRNA-mediated depletion of Cep192 inhibits 1) the motility of cancer cells and primary human keratinocytes *in vitro*; 2) matrigel invasion of primary tumor cells *ex vivo*; 3) axon outgrowth from primary neurons. This additionally identifies Cep192, over Kif19, as a therapeutic target for mitigation of pain after wounding.

[00131] The centrosome is an organelle that serves as the main microtubule organizing center (MTOC) of the animal cell as well as a regulator of cell-cycle progression. Centrosomes are composed of two orthogonally arranged centrioles surrounded by an amorphous mass of protein termed the pericentriolar material (PCM). The PCM contains proteins responsible for microtubule nucleation and anchoring.

[00132] Cep192 is a centrosome scaffolding protein required for centrosomal microtubule nucleation during mitosis (Gomez-Ferreria, Rath et al. 2007; Gomez-Ferreria

and Sharp 2008). Disclosed herein is that Cep192 is also required for the nucleation of centrosomal microtubules in interphase cells. Depletion of Cep192 strongly suppresses the motility of both cancer and skin cells and thus Cep192 is a novel target for anti-metastatic and anti-fibrotic therapeutics. Additionally, depletion of Cep192 inhibits axon outgrowth from primary adult rat dorsal root ganglion neurons. Thus, Cep192 can also be targeted to suppress excessive early axon sprouting known to be associated with pain.

[00133] Cep192 is found to localize to centrosomes in interphase cells and is required for normal microtubule organization (see Fig. 9). Cep192 was also found to stimulate microtubule nucleation from centrosomes (Fig. 10).

[00134] It was found that Cep192 is required for normal cell motility *in vitro*. Fig. 11 shows in A) time-lapse phase-contrast images of control and Cep192 siRNA treated U2OS cells from an *in vitro* wound healing assay. U2OS cells were plated into Ibidi Culture-Insert dishes following knockdown. In B), significantly fewer Cep192 depleted cells entered the wound zone relative to controls. $P<0.0001$. S.E.M. is depicted as vertical bars. Fig. 11C) shows time-lapse phase-contrast images of control and Cep192 siRNA treated HEKa (human epidermal keratinoctyes- adult) cells from an *in vitro* a wound healing assay. HEKa cells were plated into Ibidi Culture-Insert dishes following. D) Shows significantly fewer Cep192 depleted cells entered the wound zone relative to controls. $P<0.0001$. S.E.M. is depicted as vertical bars.

[00135] Depletion of Cep192 prevents tumor cell invasion and metastasis. The effects on anaplastic thyroid carcinoma are shown in Fig. 12 and the effects on large cell lung tumor are shown in Fig. 13.

[00136] Depletion of Cep192 inhibits axon outgrowth from primary rat neurons. Fig. 14 shows fidgetin and Cep192 regulate axon regeneration. Images are immunofluorescence micrographs of primary adult rat DRG neurons treated with control, Fidgetin or Cep192 nanoparticle encapsulated siRNA. Cells were fixed 24 hours after plating and siRNA treatment. Bottom right panel shows the average axon length in each condition (longest process from each individual cell was measured; error bars are SEM). ***$P<0.01$; **$P<0.05$. In contrast to Fidgetin np-si, Cep192 np-si treatments suppress axon regrowth in adult DRG neurons — Cep192 and Fidgetin are likely functionally antagonistic in this regard. Agents that suppress Cep192, such as Cep192 np-si, can be used to suppress excessive early axon sprouting known to be associated with pain.

Methods

[00137] Np-si application for experiments: This can be performed by mixing the nanoparticles in either sterile saline or water to achieve the targeted concentration in no more than 10 ul aliquots. The solution is applied directly to the wound, or target area, where it is rapidly absorbed. Controls include i) non-specific siRNA nanoparticles and ii) water or saline alone. Two different treatment regimens are exemplified here: In regimen 1, np-si are administered daily beginning 30 minutes after wounding though day 8. In regimen 2, np-si are administered every other day beginning 30 minutes after wounding (day 0, 2, 4, 6, and 8).

[00138] Np-si formulation. For the nanoparticles a hydrogel-based nanoparticle platform is used. A final concentration of siRNA of 0.30 to 0.35 nmole per mg of dry nanoparticles is used in studies. siRNAs are anionic but cationic stabilization is preferred for nanoparticle encapsulation and siRNA stability. In an embodiment, the formulation utilizes the cationic polysaccharide chitosan as a stabilizing factor for the siRNA. The cationic character of the nanoparticles can be enhanced by doping the formulation with varying amounts of positively charged amino silanes.

[00139] PEGylation of the np-si: Increasing the size of PEG molecules incorporated into the formulation may increase the rate of release for siRNA (this is determined using fluorescent labeled siRNA). Post-preparative PEGylaton of the np-si can be means of further minimizing aggregation and improving *in vivo* lifetime. In an embodiment, the conjugation of functionalized PEG chains (PEG-500/PEG-3000/PEG-5000) to the surface of np-si in alcohol/water medium to minimize the leakage of siRNA from the particles is effected.

[00140] Wound healing determination: Photographs of the wounds are taken daily to follow gross visual wound healing as assessed by the area of the wound uncovered by the migrating epithelia. Each wound is measured daily using a caliper and the area is determined.

[00141] Morphometric analysis of wound sections: Wound re-epithelialization is measured in Hematoxylin and Eosin stained sections from the center of the wound. The distance between the wound edges, defined by the distance between the first hair follicle encountered at each end of the wound, and the distance that the epithelium had traversed

into the wound, is analyzed using ImageJ. The percentage of re-epithelialization [(distance traversed by epithelium)/(distance between wound edges) x 100] is calculated and averaged for two sections per wound.

[00142] Collagen deposition: Staining is performed using Masson's trichrome stain and the percentage of blue collagen-stained area relative to the total area of the wound bed after taking digital images. This is quantified by counting the number of pixels staining above a threshold intensity and normalizing to the total number of pixels.

[00143] Proliferation rate. To visualize cell proliferation, mice are injected intrapertonially (120mg/kg BrdU (Sigma-Aldrich, USA)) 2-4 hrs. prior to sacrifice and cutaneous wounds are harvested for paraffin embedding and BrdU immunohistochemistry. Tissue sections will be deparaffinized and rehydrated through graded alcohols and incubated overnight at room temperature with a biotinylated monoclonal BrdU antibody (Zymed, South Francisco, CA).

[00144] Nuclear staining are visualized using Streptavidin-peroxidase and diaminobenzidine (DAB) and samples will be lightly counterstained with hematoxylin. Wound tissue from mice that were not injected with BrdU is used as a negative control. Digital photographs are taken at high (40-60X) magnification (Zeiss AxioHOME microscope) and epithelial cells sections are examined using ImageJ software and classified as BrdU positive if they grossly demonstrated brown-stained nuclei from DAB staining or as BrdU negative if they were blue stained. nuclei. The proliferation rate is then calculated as the percentage of BrdU positive cells over the total number of cells within the ROI.

[00145] Angiogenesis: Wound sections are stained using CD31 antibody (also called platelet-derived endothelial cell adhesion molecule-1). Digital images at 40x magnification covering the majority of the wound bed are taken and the percent area stained in each image are quantified by counting the number of pixels staining above a threshold intensity and normalizing to the total number of pixels. Threshold intensity will be set such that only clearly stained pixels are counted. Staining identified as artifact, large vessels, and areas deemed to be outside the wound bed will be excluded.

[00146] Reinnervation: Wound sections post injury days 7 and 14 will be stained for protein gene product 9.5 (PGP9.5), a pan-neuronal marker, and the sensory neuropeptides calcitonin gene related peptide (CGRP) and substance P (SP). Nerve fiber growth into the wounds is compared between control and treated wounds,

[00147] Histopathology of epidermal stem cells and the stem cell niche at the hair bulge. For identification of epidermal stem cells in various cohorts of animals, immunohistochemistry is performed for the following markers of epidermal stem cells-CD34, Cytokeratin 15, Bmi1,Lrig1, Blimp1, Nestin, Lgr5, CD-200, β1-Integrin, according to published reports. The epidermal stem cell niche is characterized by immunohistochemistry for α-smooth muscle actin (α-SMA) to detect epidermal myofibroblast and vascular smooth muscle cells, ICAM-1 for endothelial cells, F4/80 for macrophages.

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What is claimed is:

1. A method of treating metastasis or inhibiting metastasis in a subject having a cancer comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat metastasis or inhibit metastasis.

2. A method of treating metastasis or inhibiting metastasis in a subject having a cancer comprising administering to the subject an amount of an inhibitor of CEP192 or of Cep192 gene product effective to treat metastasis or inhibit metastasis.

3. A method of treating fibrosis or scarring, or of inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of KIF19 or of Kif19 gene product effective to treat fibrosis or scarring, or inhibit fibrosis or scarring.

4. A method of treating fibrosis or scarring, or inhibiting fibrosis or scarring, in a subject in need thereof comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat fibrosis or scarring, or inhibit fibrosis or scarring.

5. A method of treating pain associated with wound healing in a subject having a wound comprising administering to the subject an amount of an inhibitor of Cep192 effective to treat pain associated with wound healing.

6. The method of any of Claims 1 or 3, wherein the KIF19 or Kif19 gene product is a human KIF19 or human Kif19 gene product, respectively.

7. The method of any of Claims 2, 4 or 5, wherein the CEP192 or Cep192 gene product is a human CEP192 or a human Cep192 gene product, respectively.

8. The method of any of Claims 1, 3, or 6, wherein the inhibitor of KIF19 is an RNAi nucleic acid.

9. The method of any of Claims 2, 4, 5 or 7, wherein the inhibitor of CEP192 is an RNAi nucleic acid.

10. The method of Claim 8, wherein the RNAi nucleic acid is a siRNA directed to KIF19 or a shRNA directed to KIF19.

11. The method of Claim 9, wherein the RNAi nucleic acid is a siRNA directed to CEP192 or a shRNA directed to CEP192.

12. The method of Claim 10 or 11, wherein the siRNA is administered.

13. The method of Claim 10 or 11, wherein the shRNA is administered.

14. The method of Claim 12, wherein the siRNA is administered as a composition comprising the siRNA associated with a nanoparticle.

15. The method of Claim 13, wherein the siRNA is administered as a composition comprising the siRNA encapsulated with a nanoparticle.

16. The method of Claim 14 or 15, wherein the nanoparticle is PEGylated.

17. The method of Claim 12, wherein the siRNA is administered as a viral vector.

18. The method of Claim 13, wherein the shRNA is administered as a viral vector.

19. The method of any of Claims 1, 2 or 6-18, wherein the cancer is a thyroid, blood, bladder, breast, colorectal, kidney, lung, melanoma, ovary, pancreas, prostate or stomach cancer.

20. The method of Claim 19, wherein the cancer is an anaplastic thyroid carcinoma.

21. The method of Claim 19, wherein the cancer is large cell lung cancer.

22. The method of any of Claims 3, 4 or 6-18, wherein the fibrosis is in response to an injury.

23. The method of any of Claims 3, 4 or 6-18, wherein the fibrosis is a fibroma, pulmonary fibrosis, cystic fibrosis, hepatic cirrhosis, endomyocardial fibrosis, from a previous myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis of the lungs, a complication of pneumoconiosis, nephrogenic systemic fibrosis, Crohn's disease fibrosis, keloid fibrosis, scleroderma/systemic sclerosis of skin or lungs, arthrofibrosis or adhesive capsulitis fibrosis.

24. The method of any of Claims 3, 4 or 6-18, wherein the scarring is skin scarring, cardiovascular scarring, or neuronal scarring.

25. The method of any of Claims 5, 7, 9, or 11-18, wherein the wound is a skin wound, cardiovascular wound, or neuronal wound.

26. The method of claim 25, wherein the skin wound is a burn wound.

27. The method of Claim 24, 25 or 26, wherein the inhibitor is applied to the skin of the subject.

28. A method of identifying an anti-metastatic agent comprising contacting a nucleic acid encoding Kif19 gene product with the agent or contacting Kif19 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Kif19 gene product or inhibits activity of the Kif19 gene product, respectively, and subsequently identifying the agent as an anti-metastatic agent or not, wherein an agent that inhibits Kif19 expression or Kif19 gene product is identified as an anti-metastatic agent.

29. A method of identifying an anti-metastatic agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and

subsequently identifying the agent as an anti-metastatic agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as an anti-metastatic agent.

30. A method of identifying an anti-fibrotic agent comprising contacting a nucleic acid encoding Kif19 gene product with the agent or contacting Kif19 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Kif19 gene product or inhibits activity of the Kif19 gene product, respectively, and subsequently identifying the agent as an anti-fibrotic agent or not, wherein an agent that inhibits Kif19 expression or Kif19 gene product is identified as an anti-fibrotic agent.

31. A method of identifying an anti-fibrotic agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and subsequently identifying the agent as an anti-fibrotic agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as an anti-fibrotic agent.

32. A method of identifying a pain-relieving agent comprising contacting a nucleic acid encoding Cep192 gene product with the agent or contacting Cep192 gene product with the agent and determining if the agent inhibits expression of the nucleic acid-encoded Cep192 gene product or inhibits activity of the Cep192 gene product, respectively, and subsequently identifying the agent as a pain-relieving agent or not, wherein an agent that inhibits Cep192 expression or Cep192 gene product is identified as a pain-relieving agent.

33. The method of any of Claims 28-32, wherein the agent is a small organic molecule, a peptide, a nucleic acid, an oligonucleotide, an antibody, an antigen-binding fragment of an antibody or an aptamer.

34. An inhibitor of KIF19, or of Kif19 gene product, for treating metastasis or inhibiting metastasis in a subject having a cancer.

35. An inhibitor of CEP192 or of Cep192 gene product, for treating metastasis or inhibiting metastasis in a subject having a cancer.

36. An inhibitor of KIF19, or of Kif19 gene product, for treating fibrosis or scarring in a subject in need thereof.

37. An inhibitor of CEP192 or of Cep192 gene product, for treating fibrosis or scarring in a subject in need thereof.

38. An inhibitor of CEP192 or of Cep192 gene product, for treating pain associated with wound healing in a subject.

39. The inhibitor of any of Claims 34-38, wherein the inhibitor is an RNAi nucleic acid.

40. The inhibitor of any of Claims 34-39, wherein the inhibitor comprises an siRNA.

41. The inhibitor of any of Claims 34-39, wherein the inhibitor comprises an shRNA.

42. The inhibitor of Claims 40 or 41, wherein the siRNA or shRNA is directed against CEP192.

43. The inhibitor of Claims 40 or 41, wherein the siRNA or shRNA is directed against KIF19.

METHODS AND COMPOSITIONS TO INHIBIT METASTASIS AND TO TREAT FIBROSIS AND TO ENHANCE WOUND HEALING

ABSTRACT OF THE DISCLOSURE

Methods and compositions are provided for inhibiting or treating metastasis based on discoveries regarding Kif19 and Cep192. Methods and compositions are provided for enhancing wound healing, treating fibrosis, reducing scarring and treating nerve pain.

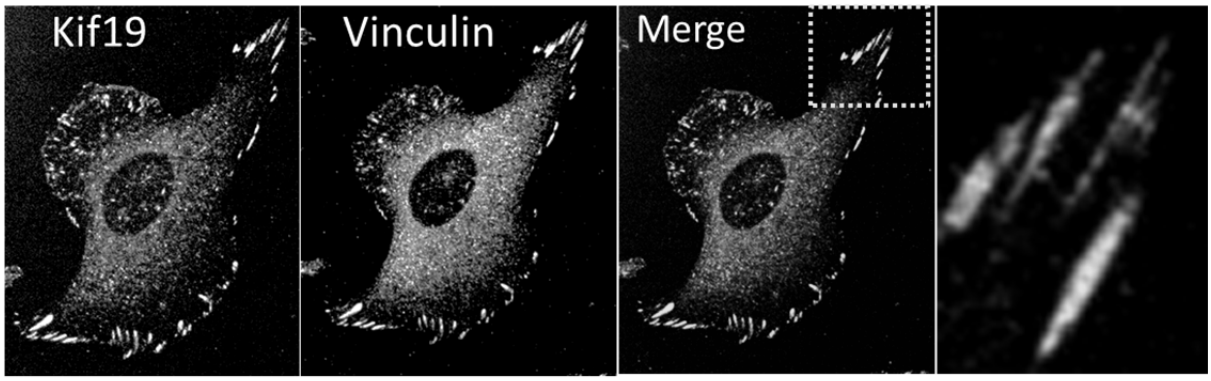

Fig. 1

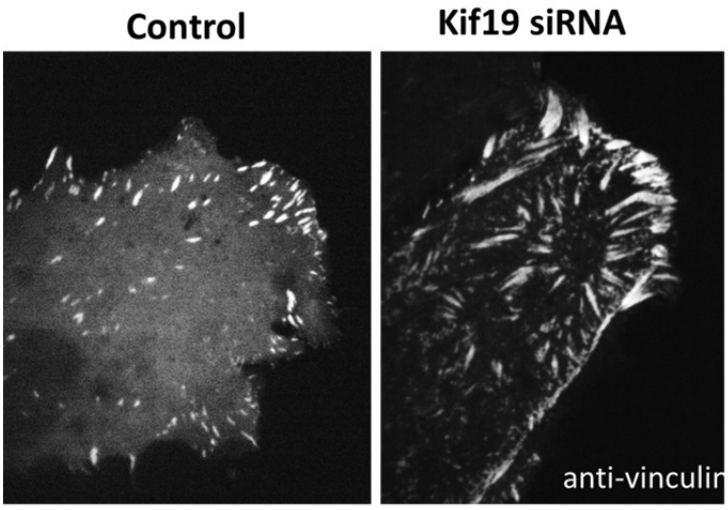

Fig. 2

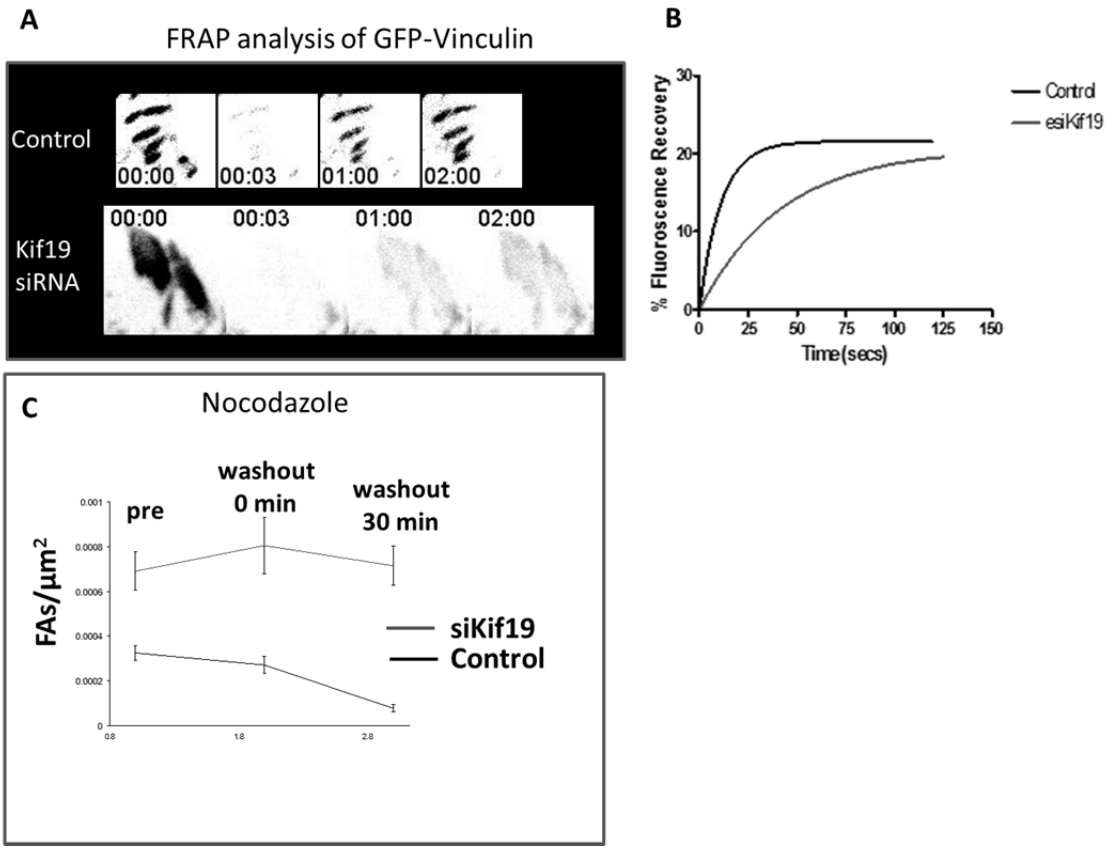
A
FRAP analysis of GFP-Vinculin
Control
00:00
00:03
01:00
02:00
Kif19
siRNA
00:00
00:03
01:00
02:00
B
% Fluoroscence Recovery
Time(secs)
Control
esiKif19
C
Nocodazole
pre
washout
0 min
washout
30 min
FAs/µm²
siKif19
Control

Fig. 3A-3C

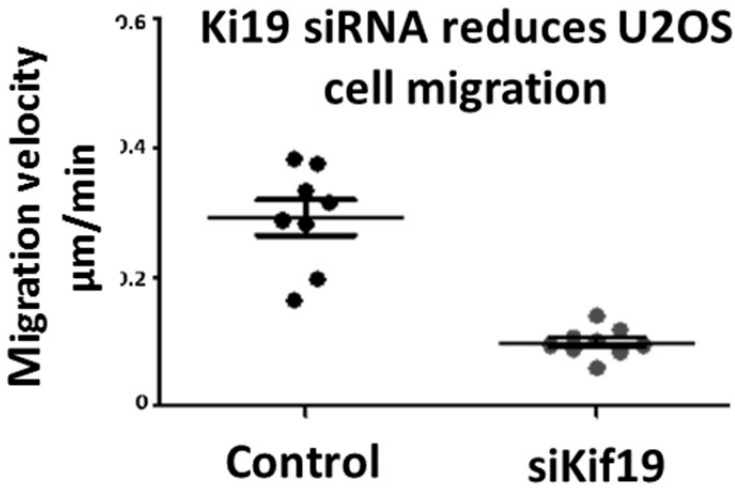
Ki19 siRNA reduces U2OS cell migration
Migration velocity µm/min
0.6
0.4
0.2
0
Control
siKif19

Fig. 4

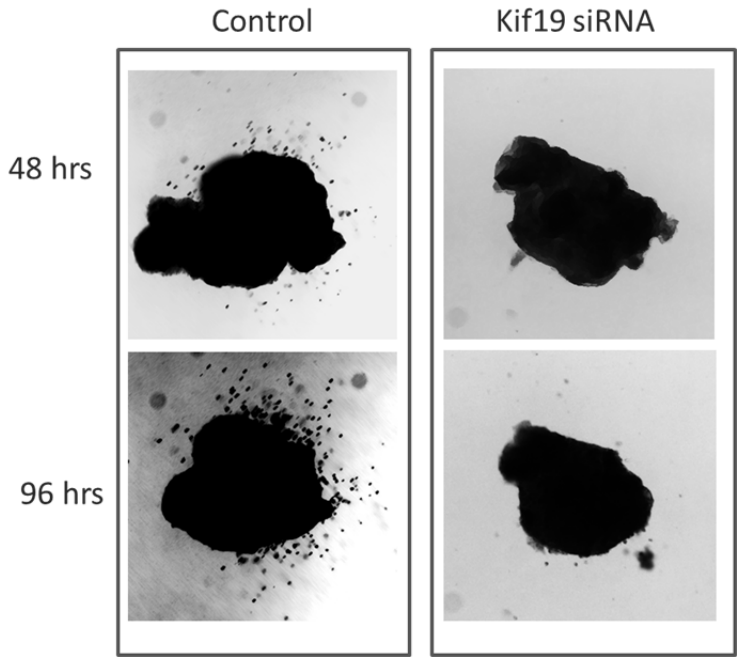

Fig. 5

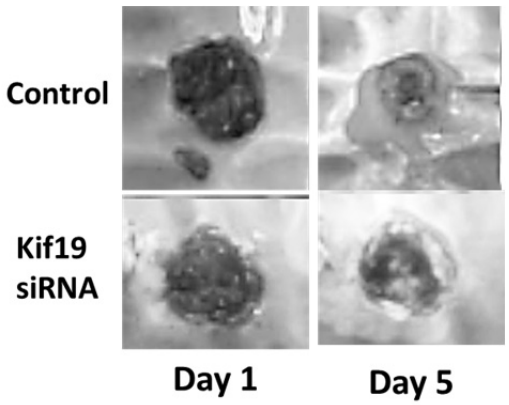

Fig. 6

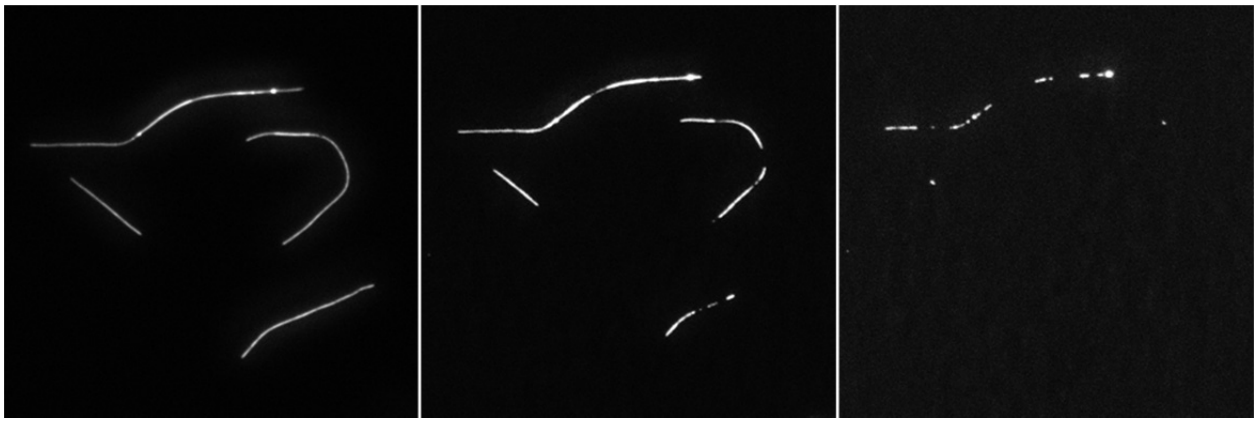

Fig. 7

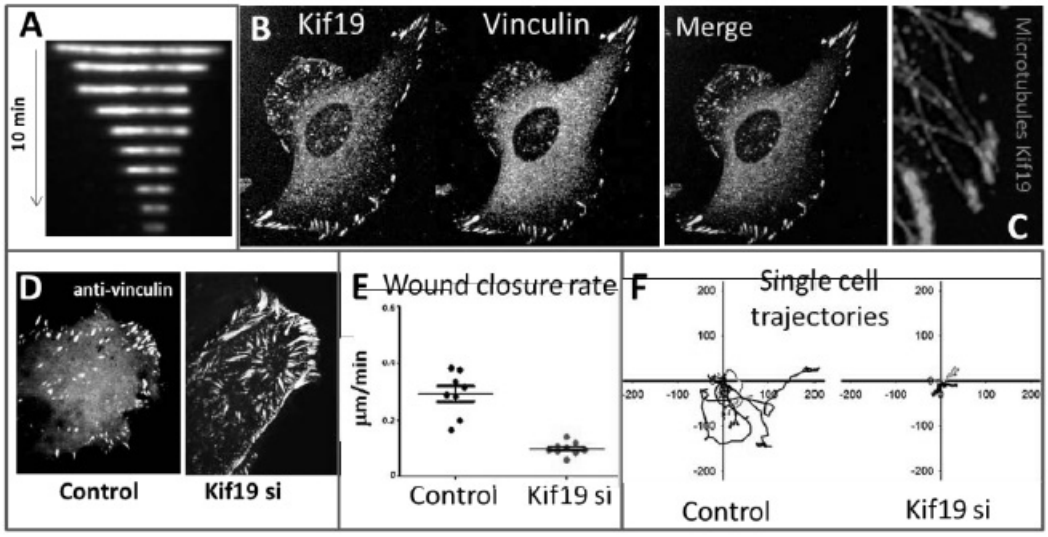
A
10 min
B Kif19
Vinculin
Merge
Microtubules Kif19
C
D anti-vinculin
Control
Kif19 si
E Wound closure rate
µm/min
Control
Kif19 si
F Single cell trajectories
Control
Kif19 si

Fig. 8A-8F

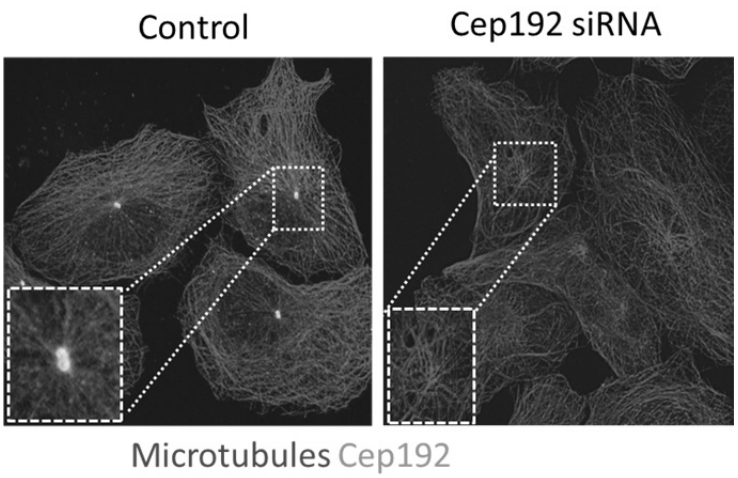

Fig. 9

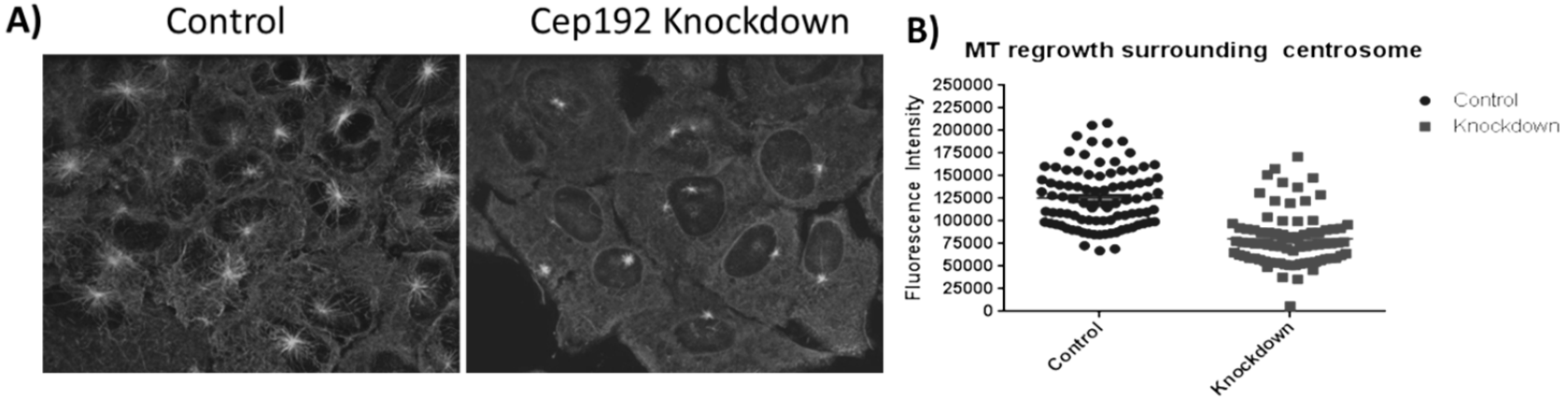
A)
Control
Cep192 Knockdown
B)
MT regrowth surrounding centrosome
Fluorescence Intensity
250000
225000
200000
175000
150000
125000
100000
75000
50000
25000
0
Control
Knockdown
Control
Knockdown

Fig. 10A-10B

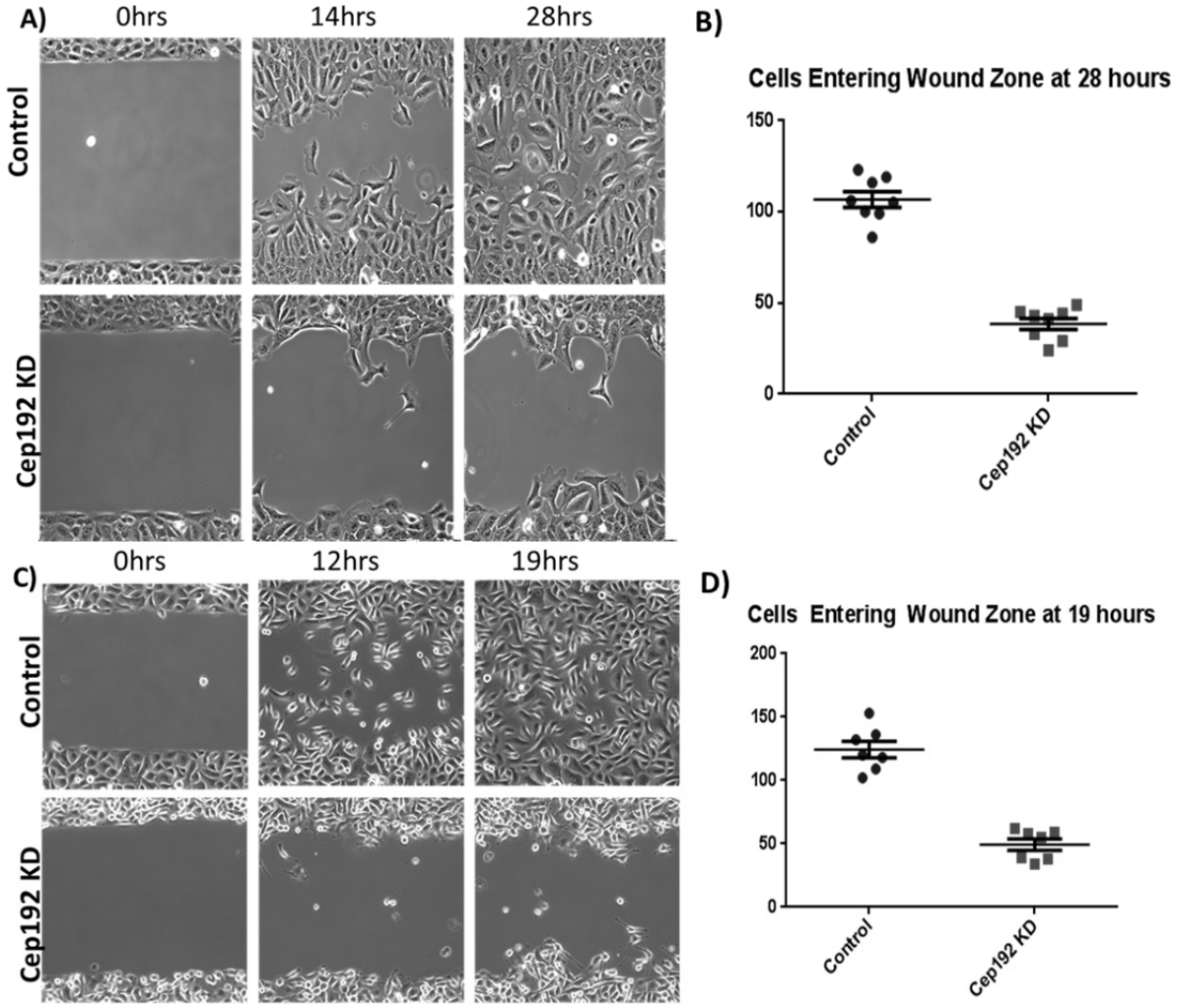
A)
0hrs
14hrs
28hrs
Control
Cep192 KD
B)
Cells Entering Wound Zone at 28 hours
150
100
50
0
Control
Cep192 KD
C)
0hrs
12hrs
19hrs
Control
Cep192 KD
D)
Cells Entering Wound Zone at 19 hours
200
150
100
50
0
Control
Cep192 KD

Fig. 11A-11D

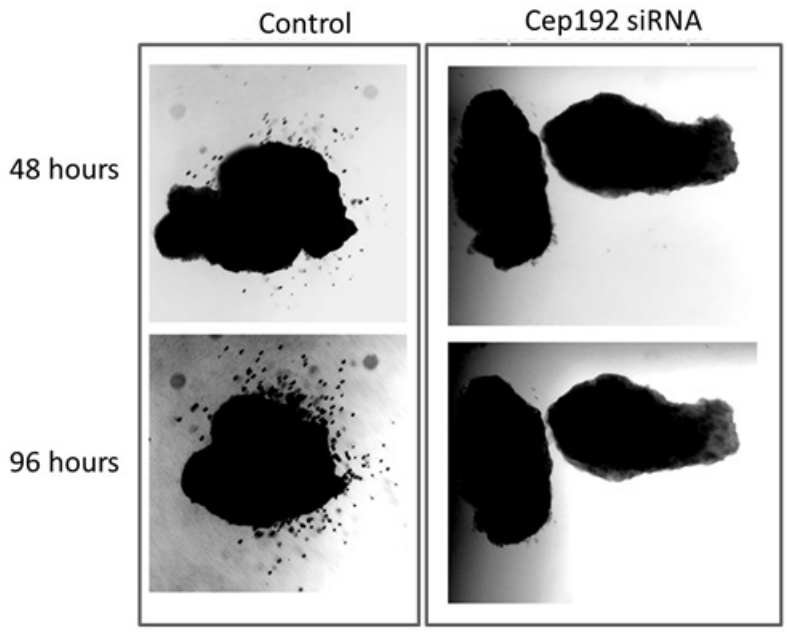

Fig. 12

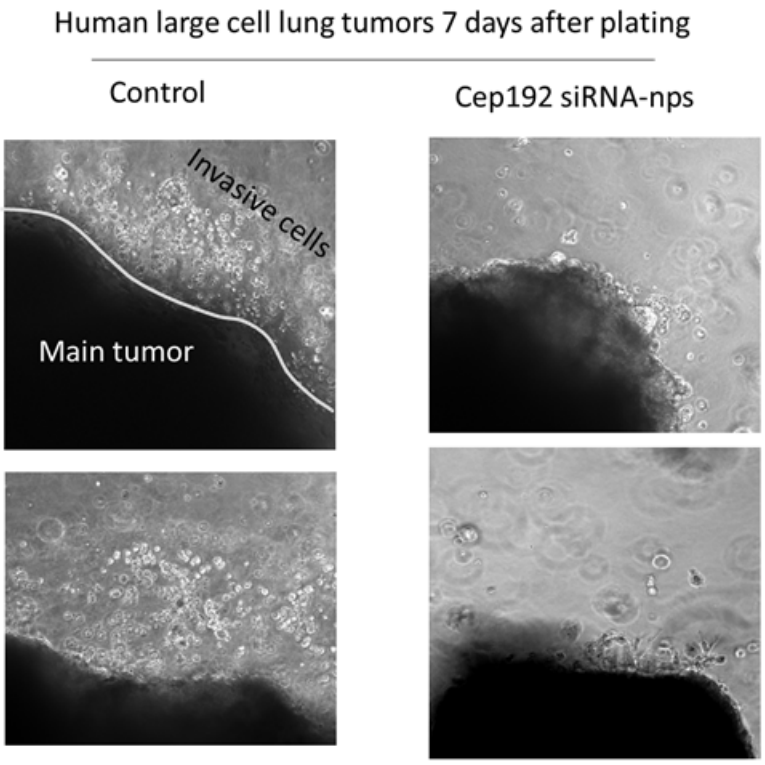
Human large cell lung tumors 7 days after plating
Control
Cep192 siRNA-nps
Invasive cells
Main tumor

Fig. 13

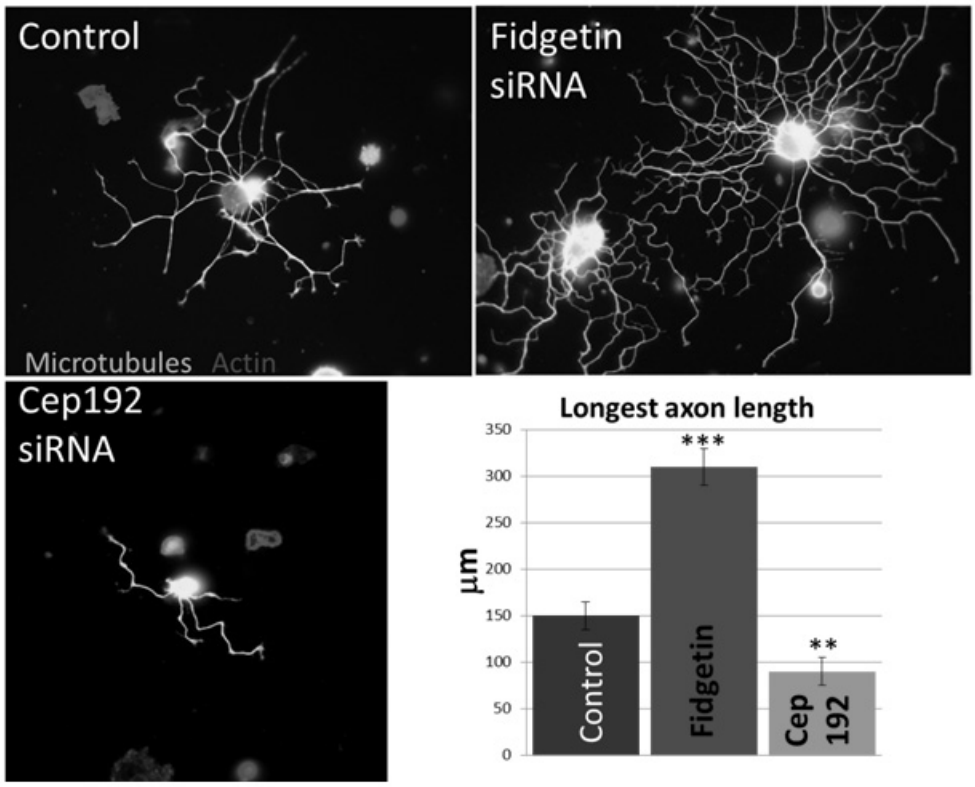
Control
Microtubules Actin
Fidgetin siRNA
Cep192 siRNA
Longest axon length
µm
350
300
250
200
150
100
50
0
***
**
Control
Fidgetin
Cep 192

Fig. 14